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SOME PROBLEMS OF THE EVOLUTION OF STARS

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To be read before the Academy, April, 1949

During the past few years several stimulating articles have appeared outlining a new theory of the evolution of stars.[1] The purpose of this note is to present certain considerations regarding the evolution of close binary stars and to discuss them in the light of the more general theories, especially that of v. Weizsaecker. The earlier theories of double-star formation are so well known that they need not be reviewed in this place. The most convincing arguments in favor of a fission process have probably been advanced by James Jeans, who in many respects continued the earlier work of George Darwin. However, serious doubts regarding the possibility of fission were expressed many years ago by F. R. Moulton, and the most potent considerations against the classical fission theory were published about fourteen years ago by G. P. Kuiper.[2] The problems of cosmogony are so involved and difficult that it is important to record the considerations of all those who have worked on this subject. Most investigators are primarily influenced by the results obtained within the scope of their own investigations. This leads me to call attention to certain observational results which are not very widely known but which have an important bearing upon all cosmogonical speculations. I am referring here particularly to the results obtained from studies of stellar rotation.

V. Weizsaecker's recent theory is essentially based upon his earlier work on the effect of turbulence in diffuse stellar objects which, in keeping with the terminology of Spitzer and Whipple, may be designated as protostars. These protostars are believed to have originated through the condensation of interstellar clouds of gas and smoke, very much as has been recently suggested by Spitzer[3] and Whipple.[4] Turbulent motions in the original medium would have produced large angular momenta in most of these protostars, and this presumably accounts for the high frequency of rapidly rotating stars of the earlier spectral types which, according to this theory, are relatively young. As the result of turbulent motions within the proto-

star, whose rotation is not that of a rigid body, angular momentum is transported from the interior regions to the outside, and finally the mass breaks into two, the nucleus retaining only a small amount of angular momentum while the outer fringe of the diffuse, nebulous mass is dispersed into space, carrying away in the process the excess angular momentum. In this manner v. Weizsaecker explains the gradual loss of mass in a hot main-sequence star and the absence of large rotational velocities in the stars of later spectral types.

However, it is now known from the work of H. Shapley[5] that although the overwhelming majority of the cooler stars of the main sequence in the Hertzsprung-Russell diagram possess only a very small amount of angular momentum there are in existence a large number of binaries of late spectral type which possess rapid rotations and large amounts of orbital angular momentum. Shapley has shown that these so-called W Ursae Majoris binaries are more numerous in space than all the other variable stars now recognized. Although he does not give a quantitative estimate of the fraction of solar type stars which are binaries of this kind it is clear that they are sufficiently numerous to require consideration when we examine the evolution of the stars in the main sequence.

A W Ursae Majoris binary normally has a spectral type close to that of the sun. Its surface temperature is therefore in the vicinity of 6000°. Each system consists of two components having masses of the order of 1 solar mass and $^1/_2$ solar mass, respectively. The diameters of these stars are somewhat smaller than those of the sun. They revolve almost in contact and they are surrounded by a common gaseous envelope which is unsymmetrical in thickness and in other physical properties.[6] The average orbital velocities of the approximate geometrical centers of the two components with respect to the center of gravity of the whole system are 110 km./sec. and 220 km./sec. Since the system rotates approximately as a rigid body the relative velocity of the points of greatest separation on the two stellar ellipsoids is at least 400 km./sec. Hence, if in the course evolution such a star should ever combine to form a single object, its rotational velocity would be considerably more than 200 km./sec. at the equator. Such a velocity would be easy to observe. Yet among the single stars of appropriate spectral type there are no objects having anything like this kind of rotation.

It might be asked what kind of star would result from the coalescence of the two components. There are all reasons to think that the process would be a fairly gradual one so that any disturbance in the constitution of the object would not for very long remove that star from the main sequence. The existence of a common envelope makes it, even now, difficult to decide whether they are single stars with two condensations, or real binaries. At any rate, if the two stars should combine without losing

mass they would form a single object having a mass of about 1.5 times the mass of the sun. If such a star remained on the main sequence, its spectral type would be late F or early G and observations show that there are no single stars within these spectral subdivisions which have the required amount of angular momentum. If the star should be disturbed to such an extent as to depart from the main sequence it would have to fall somewhere along the appropriate line for mass 1.5 ⊙ in the H-R diagram. Otherwise it would be necessary to suppose that it would fail to obey the mass luminosity relation and this would place it in a portion of the H-R diagram where there are so few stars per unit volume of space that we can safely exclude the possibility. As a matter of fact this argument strongly suggests that the very numerous W Ursae Majoris type binaries do not

TABLE 1

SYSTEMS OF THE TYPES OF W URSAE MAJORIS

NO.*	STAR	SP.	P, DAYS	KM./SEC. γ	KM./SEC. K_1	KM./SEC. K_2	m_1	m_2	a	INT.	J_1/J_2
1	W UMa	F8	0.334	− 5	134	188	0.9⊙	0.6.	1.4	Var.	<1
2	TX Cnc	F8	0.383	+36	112	217	1.5	0.8	1.9	Var.	<1
3	44i Boo B	G2	0.268	+ 3	116	232	1.0	0.5	2.0	?	<1
4	ER Ori	G2	0.423	+35	95	155	0.6	0.4	1.6	?	>1
5	YY Eri	G5	0.321	−20	130	200	1.0	0.7	1.5	Var.	<1
6	VW Cep	KO	0.278	−35	75	230	1.1	0.35	3.1	Var.	<1
7	AH Vir	KO	0.408	+10	105	250	1.5	0.7	2.4	Var.	<1
8	RZ Cam	KO	0.339	−12	130	270	1.6	0.8	2.1	Var.	<1
9	V 502 Oph	G2 + F9	0.453	−37	95	235	1.2	0.5	2.5	?	<1
10	SW Lac	G3	0.321	−22	172	202	0.5	0.4	1.2	Var.	<1
	mean				110	220	1.00	0.5	2		

* References: 1. Joy, *Astrophys. J.*, **49**, 189 (1919); Struve and Iwanowska (unpublished); 2. Popper, *Ibid.*, **108**, 490 (1948); 3. Popper, *Ibid.*, **97**, 407 (1943); 4. Struve, *Pubs. Astron. Soc. Pacific*, **56**, 34 (1944); 5. Struve, *Astrophys. J.*, **106**, 92 (1947); 6. Popper, *Ibid.*, **108**, 490 (1948); 7. Chang, *Ibid.*, **107**, 96 (1948); 8. Struve and Gratton, *Ibid.*, **108**, 502 (1948); 9. *Ibid.*; 10. Struve, *Ibid.* (in press).

have descendant single stars whose mass is 1.5 ⊙. Similarly we can also show that the reverse process is not possible because none of the single stars of mass 1.5 ⊙ has enough angular momentum to produce a binary of the W Ursae Majoris type.

I believe that this calls for a modification of v. Weizsaecker's theory. Otherwise the W Ursae Majoris type binaries would remain unexplained and they are altogether too numerous to be ignored.

Spectroscopic observations are now available for ten of these objects. The pertinent data are summarized in table 1.

The third column of the table gives the spectral type which is the same for both components of each system except in the case of V 502 Ophiuchi. In that star the more massive component is of spectral type G2 and the

less massive of type F9. The fourth column gives the period in days. It ranges from 0.268 day to 0.453 day. The fifth column gives the radial velocity in km./sec. of the center of gravity of each system. The ten individual values clearly reflect the motion of the solar system through the local group of stars and in addition shows that the individual peculiar motions of these binaries are distributed approximately as they are among single stars of similar spectral types and luminosities. The sixth and seventh columns give the orbital velocities of the two components in km./sec. with respect to the center of gravity. Columns 8 and 9 list the masses of the two components in units of the mass of the sun. The quantity α is the mass ratio. The next to the last column shows whether the spectral lines of the fainter component undergo variations in intensity and the last column shows whether the ratio of the surface brightnesses of the more massive component to the less massive component is smaller than 1 or greater than 1. Attention should be called to the fact that the spectroscopic observations give us only the mass functions of the two components $m_1 \sin^3 i$ and $m_2 \sin^3 i$. The inclinations are either known from the photometric observations or they can be roughly estimated, but there is some uncertainty attached to these values of i. Hence the tabular masses of the two stars are not always known with great precision, but they are known sufficiently well to give us a general idea of the properties of these systems. On the contrary, the mass ratio α is known with great precision since it is equal to the ratio K_2/K_1.

Of special interest is the remarkable tendency of these systems to show spectral lines of variable intensity. As a general rule the approaching component is relatively stronger than the receding component. There are indications that the amount of variation is variable in time, but as yet no periodicities have been found. It is very probable that these changes are related to peculiar changes in the light curves of these variables which have recently been discussed by Eggen.[7] The two maxima of the light curves, which correspond to the times of greatest elongation of the two components, are not of the same height; and the amplitude of this variation, which can be represented by a term in $\sin \theta$, where θ is the longitude in the orbit counted from primary minimum, is variable between zero and a small negative value. The exact connection between the photometric and spectrographic results is not yet known but, generally speaking, the system is less luminous when the less massive component is approaching and has its greatest absorption intensity. This phenomenon cannot be adequately explained as the result of ordinary differences in the temperatures of the advancing and receding hemispheres of the two stars and it must be somehow caused by the stream of gas which forms the common envelope.

Exactly the same spectroscopic phenomenon was found many years

ago in spectroscopic binaries of very early type. It is most conspicuous in J. S. Plaskett's star of large mass, HD 47129, and it is also present in AO Cass, another very massive star of spectral type O. There can be no doubt that the phenomenon is the same and that in these two otherwise very different groups of binary systems we observe the effect of gaseous masses whose optical thickness is greatest over the advancing hemisphere of the less massive component of each system and perhaps also over the advancing hemisphere of the more massive component. The origin of these gaseous masses can only be guessed by analogy with several other binaries, especially UX Mon. I am inclined to believe that they may be caused by vast prominences which not only produce the variation in the line intensities but also give rise to the gaseous streams which influence the systems and produce various spectroscopic effects, such as double emission lines in numerous binaries of early spectral type and peculiar absorption features in other systems, which distort the velocity curves of these objects.

Some time ago I made a crude estimate of the amount of the material contained in such a ring-like gaseous formation. The result was $10^{-8}\ \odot$. No doubt, these streams of gas are secularly unstable. In the earlier and hotter systems radiation pressure must rapidly drive them out into space. We actually observe this phenomenon in β Lyrae. Even if we assume that the rate of dissipation of the gaseous envelope results in its complete renewal once per year the original mass of the star would be exhausted in something like 10^{+8} years. This rate of dissipation would require an average velocity of expansion of only about 5×10^{-2} km./sec. A rate of expansion 200 times faster, or 10 km./sec., would still be consistent with the absence of any conspicuous velocities of expansion in most systems (but in Plaskett's star the observed rate of expansion is about 100 km./sec. and in β Lyrae it is of the order of 50 km./sec.). It is thus entirely possible that the mass of an early-type system would be nearly exhausted in an interval of 10^6 years.

It is remarkable that v. Weizsaecker's estimate based upon his ideas of turbulence leads to precisely the same interval of time for the dissipation of mass and rotational momentum in a protostar of early spectral type and of an equatorial velocity of rotation of the order of 100 km./sec.

This leads me to suggest that perhaps the W Ursae Majoris systems have been formed out of the binaries of early type, such as β Lyrae, U Cephei, or Plaskett's massive star. I recognize that this hypothesis has certain weaknesses. For example, there is the difference in the galactic concentration of the early-type binaries and the W Ursae Majoris systems and also the prevalence of larger individual space motions among the latter group. However, this difficulty is common to the present hypothesis

as well as to v. Weizsaecker's original theory. It requires the assumption that in the course of 10^6 or 10^8 years the distribution of the stars in the galaxy and the approach to equipartition of the translational energies has been sufficiently accomplished and this would presumably require a much greater density of stars in the past than is observed at the present time. There is also the difference in the space frequencies of the early-type binaries and the binaries of the solar type. I am disregarding here those binaries which contain super-giant components. They are so rare in space as not to influence the arguments appreciably. The relative frequencies of close binary systems among the stars of types O, B and A with respect to the single stars of the same types is probably very nearly the same as for the W Ursae Majoris systems and the single stars of types: late F, G and K; but per unit volume of space the early-type stars are at least 50 times less frequent than are the late-type stars. Hence, the same ratio must apply also to the two groups of binaries. If the early-type binaries gradually lose mass and become W Ursae Majoris binaries it is necessary to assume that the length of time they remain in the latter stage greatly exceeds that which they require in their early-type stage. This argument again is not limited to my suggestion but is common also to v. Weizsaecker's theory, and it may be related to the relatively great youth which the Bethe theory of stellar energies attributes to all stars of very early type.

One further argument which suggests that the early-type binaries represent the youngest stage of double-star formation may perhaps be seen in the fact that those systems which, like U Cephei, consist of an early-type component of small size and a late-type component of large size show large departures from the mass luminosity relation for the fainter components: these sub-giants are much too luminous for their masses. It almost seems as though the less massive component in a recently formed binary system has been seriously disturbed from equilibrium and has wandered off the main sequence in the direction of higher luminosities. We cannot trace their course in the conventional H-R diagram because these stars do not obey the mass luminosity relation, but I think we can reasonably conclude that they are anomalous, even though the more massive early-type components are still on the main sequence. What happens when a star divides into two approximately equal components is not certain, because the empirical mass-luminosity relation at its upper end is based upon these systems. But it is significant that such stars as HD 47129 and AO Cass show spectroscopic features of very high luminosity. Unsöld and especially Weizsaecker have called attention to the possibility that some catastrophic phenomenon, such as a super-nova, results in the process, but perhaps this is carrying us too far since it is not necessary for our purpose to discuss whether some modernized version of the fission theory involving the interplay of nuclear reactions in the stellar

interiors might meet the objections which Kuiper had made to the classical fission theory of Jeans.

One other point remains to be considered. What happens to the W Ursae Majoris binary if it continues dissipating mass and angular momentum through the medium of the common envelope, which in many respects would play the rôle of v. Weizsaecker's nebulosity? Perhaps what would result is precisely what v. Weizsaecker has predicted: a system of planets and a rapidly dissipating nebulosity which carries off most of the rotational momentum. If this is possible then it is perhaps not too far-fetched to think of the W Ursae Majoris systems as being the parent bodies of what we now observe as single solar-type stars without much angular momentum.

Despite its obviously bizarre appearance, this hypothesis has the attractive feature of giving us some observational evidence of precisely the kind of nebulosity which v. Weizsaecker requires. Otherwise, within the domain of main-sequence stars later than about type A, shell-like formations are not known, at least not among the single stars. Yet one would expect to observe various kinds of dilution effects in a mass of gas having the properties of the v. Weizsaecker nebulosity.

[1] V. Weizsaecker, C. F., *Z. Astrophys.*, **24**, 181 (1947); Unsöld, A., *Ibid.*, **24**, 278 (1948); Ambartzumian, A., "The Evolution of Stars and Astrophysics" (in Russian), *Acad. Sci. Armenian S.S.R.*, 1947.

[2] Kuiper, G. P., *Pubs. Astron. Soc. Pacific*, **47**, 15, 121 (1935).

[3] Spitzer, L., Jr., "Centennial Symposia Volume," *Harv. Obs. Monographs*, No. 7, page 87 (1948); "Physics Today," **1**, No. 5, 6 (September, 1948).

[4] Whipple, F., "Centennial Symposia Volume," *Harv. Obs. Monographs*, No. 7, page 109 (1948).

[5] Shapley, H., "Centennial Symposia Volume," *Ibid.*, page 249.

[6] Kuiper, G. P., *Astrophys. J.*, **108**, 541 (1948); Struve, O., *Ann. d'Astrophysique*, **11**, 117 (1948).

[7] Eggen, O. J., *Astrophys. J.*, **108**, 15 (1948).

THE ACTION OF ALLELIC FORMS OF THE GENE A IN MAIZE II. THE RELATION OF CROSSING OVER TO MUTATION OF A^b *

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Communicated by M. M. Rhoades, February 15, 1949

Inherited changes other than those which may be regarded as true mutations of the gene occur frequently in plant and animal material. Among such changes in *Drosophila* some are associated with crossing over

between intrachromosomal duplications.[1-3] These latter may be distinguished from chromosomal aberrations of a more gross nature since the changes are localized within small regions and the duplicated segments involved carry either the same allele or factors closely related in their effects. It has been suggested that this highly localized duplication of chromatin followed by mutation and selection may constitute an important source of new genes. Further, depending on its frequency of occurrence, this phenomenon has a direct bearing on the interpretations of studies inquiring into the manner of action of genes. These studies assume, as the simplest situation, that each member of an allelic series is a single unit of action. If this assumption is maintained in those cases in which the effects of alleles are non-linear it becomes necessary to consider the action of this single unit as correspondingly more complex from a physiological viewpoint. However, if it can be demonstrated that the alleles in question are represented on the chromatin thread by separable entities it is possible to view their action according to schemes which are not so complex from the standpoint of action of the individual unit.

Evidence is presented here for the compound nature of the A^b gene, a member of the relatively extensive A series of alleles in maize. As noted in the original report,[4] in its determination of brown pericarp this allele is dominant to the wild type A, which is associated with red pigmentation. Since A itself is dominant to recessive a (brown pericarp) the dominant effect of A^b is not expected on the basis of any simple scheme representing the action of these alleles. From a study[5] of the dosage and dominance effects of certain mutants derived from A^b and designated A^d it is known that these alleles retain the distinctly antimorphic effects of the parent allele; their action is unexplained on a simple basis.

A^b mutates spontaneously with high frequency to alleles of lower level. Measured in the female germ line, Stadler[6] found a rate of 0.0005 (19: 36,661) for mutation to the A^d allele. Among 55,765 tested female gamates, no mutations of A^b to a were found. However, mutations to a occur indirectly. A^d, itself a mutant derivative of A^b, has produced 4 mutations to a (4: ca. 50,000).[7] It appears from this that either the mutation of A^b is highly directed or this gene may be compound. In the event of the latter the production of the mutant a by two successive changes, but never in a single step, is explained if it is assumed that two components of A^b are independent in their mutation.

To test the possibility that A^b is represented by closely linked determinants experiments were undertaken to determine whether there is a relation between mutation of this allele and crossing over in or near the A locus. For such an experiment it is desirable to study mutation in $A^b\,A^b$ plants heterozygous for marker genes which are closely linked and located on either side of A^b. If a choice of marker genes can be made such that only

a small segment of the chromosome lies between them, multiple exchanges in this region may be eliminated or greatly reduced. Under these conditions the frequency of strands which experience multiple exchanges in the marked segment but which appear as non-crossover products is negligible. Where the distance between marker genes is greater the efficiency of the experiment is reduced in proportion to the frequency of double crossover strands which appear as non-crossovers. Unfortunately, until very recently there were no known mutant genes showing close linkage with *A*. At the time the present experiments were begun the best choice of markers was that of lg_2 and *et*, which lie, respectively, 33 units to the left and 13 units to the right of *A* in the long arm of the third chromosome. Since the recombination value between *lg* and *et* is high (ca. 42%), it was considered that even in the event that mutations of A^b were always associated with crossing over many of the mutants would appear as non-crossovers owing to the high frequency of double exchanges between *lg* and *et*. This disadvantage may be lessened if plants of the constitution $A^b a$ are employed instead of the homozygotes. This substitution allows the use of *a* as a third marker gene in the experiment; instead of 42 units of recombination the values corresponding to the *lg-a* and *a-et* intervals may now be employed. Since mutants of the A^d type have never been obtained from *a*, it is certain, after suitable testing, that those which occur in these experiments have originated from A^b. Moreover the A^d mutant seeds which are pale in appearance are distinguishable from both the deep purple seeds associated with A^b and the colorless phenotype associated with *a*.

The data reported here are derived mainly from one type of cross: $A^b/lg\ a\ et \times lg\ a\ et$. Plants having these constitutions were grown at Princeton in the summers of 1947 and 1948. In order to avoid the occurrence of pale seeds due to contaminating pollen grains the plants were started and reached the flowering stage earlier than any other families in the field; moreover the mass pollination technique was discarded in favor of individual hand pollinations. The A^d mutants from the 1947 experiments have had two generations of testing since their occurrence. These will be considered separately from those obtained in 1948 since the latter have not been as thoroughly tested.

Approximately half of the seeds produced by this cross have deep purple aleurones ($A^b\ a\ a$) and half are colorless ($a\ a\ a$). Ears of two families which produced a total of 27,936 purple seeds in 1947 were examined for the occurrence of pale seeds. Fifty-one pale seeds (suspected A^d mutants) were obtained for testing. Of these, 36 had etched endosperms (*et et et*) and 15 were normal (*Et et et*). Not all were expected to be true mutants since these included many seeds which deviated only slightly in phenotype from the purple class. Unfortunately only twenty of the 51 cases survived beyond the seedling stage. Thirteen of these were indistinguishable at

maturity from sib $A^b\ a$ plants grown as controls. That these plants carried an unmutated A^b gene is established from their crosses with sib $A^b\ a$ plants. If the plants in question had carried a mutant A^d gene ($A^d\ a$) three types of seeds would have resulted from these crosses: purple, pale and colorless. Only purple and colorless seeds were produced.

The remaining seven plants of the twenty which grew to maturity had a reddish brown plant color in distinct contrast to the purple phenotype of sib $A^b\ a$ plants. That these plants carried a mutated allele of A^b producing an intermediate phenotype is confirmed by their selfed and outcrossed progenies. These data are summarized in table 1.

Six of the seven plants yielded progenies following self pollination. As indicated in the table only pale and colorless seeds were produced on these ears. Crosses of each of the pale plants with $A^b\ a$ individuals gave purple, pale and colorless seeds. From this it is apparent that all seven of the pale plants were heterozygous for a mutant gene associated with intermediate plant and aleurone phenotype and originating in the female germ line of the $A^b\ a$ plants of the original cross.

TABLE 1

SUMMARY OF DATA ON ALEURONE COLOR FROM SELFED AND CROSSED PROGENIES OF THE SEVEN PALE PLANTS ORIGINATING FROM THE CROSS: $A^b/lg\ a\ et \times lg\ a\ et$

	SELFED PROGENIES			CROSSES WITH $a\ a$		
PLANTS	PURPLE	PALE	COLORLESS	PURPLE	PALE	COLORLESS
1A-1	0	125	48	0	788	866
8A-1	0	94	49	0	469	472
9A-1	0	68	26	0	148	144
14A-1	0	146	46	0	434	465
19A-1	0	40	14	0	693	695
78-1	0	76	18	0	143	159
78H-1	..	...	..	0	360	365

Some of the *lg a et* plants used as a source of pollen in the 1947 crosses carried *Dt*. This gene induces the mutation of *a* to *A* and to other alleles of higher level. Rhoades[8,9] has shown that the frequency of this change is conditioned by the number of doses of both the *a* and *Dt* genes. The *a a a Dt dt dt* endosperms of seeds resulting from crosses with pollen which transmitted *Dt* showed a moderate degree of dotting on a colorless background. The cells of the purple endosperms ($A^b\ A^b\ a\ Dt\ dt\ dt$) carry both *a* and *Dt* and presumably some of these carry an *A* gene resulting from the *Dt*-induced mutation of *a*. However, no dots have been observed on purple endosperms of this constitution and it may be inferred that the mutant cells ($A^b\ A^b\ A$) are indistinguishable from $A^b\ A^b\ a$ cells. This failure of the mutated *A* to modify the phenotype of cells which already carry A^b has an application in establishing with certainty the mutant character of the pale seeds suspected of carrying an A^d allele. If the pale

phenotype of these seeds is owing to the action of modifier genes and not to a change in the A^b gene they as well as any pale seeds in their progeny are expected to be without dots. If, however, a mutation of A^b to A^d is involved, such that the endosperms are of the constitution $A^d\ A^d\ a\ Dt\ dt\ dt$ the cells carrying the *Dt*-induced, mutant *A* gene ($A^d\ A^d\ A$) are expected to appear as purple dots on a pale background. Three of the seven pale seeds whose progeny test are given in table 1 had dotted endosperms. Two other seeds, though they had no dots, carried *Dt*. The absence of dots on these seeds may be attributed to the fact that dots frequently are absent on seeds which carry single doses of *Dt* and *a*. Each of the selfed progenies from these five seeds produced many pale, dotted endosperms. In a later generation all seven mutant cases were tested for the presence of dots on pale background by crossing the $A^d\ a$ plants with $a\ a\ Dt\ Dt$ individuals. In all cases the presence of pale seeds with dots confirms the changed nature of the A^d gene.

In view of the fact that the A^d mutants originated in $A^b\ a$ plants it is necessary to rule out the possibility that they were derived from *a*. In a previous study[5] it was established that a number of mutants of the A^d type which could have come only from A^b share several distinctive types of effect: (1) These mutants, like the parent A^b allele, are dominant to *A* in their brown pericarp effect; (2) though the mutants are associated with a decidedly intermediate plant and aleurone phenotype, there is no cumulative effect with increasing doses; (3) in compounds with an allele of a higher level of action the A^d mutants detract from, rather than add to, the effect of the former. Since mutants having this combination of effects have never been obtained from *a*, a study of the seven A^d mutants under consideration may be expected to establish conclusively their origin. The original seven plants having the constitution $A^d\ a$ were crossed with $A\ A$ individuals. The pericarp color of the F_1 plants indicated, for each of the seven cases, that the A^d gene is dominant to *A* in its effect on this tissue. To test the effects of the A^d mutants in compounds with stronger acting alleles the $A^d\ a$ plants were crossed with $A^{br}\ A^{br}$ individuals. In the F_1 progenies plants having the constitutions $A^{br}\ A^d$ and $A^{br}\ a$ were compared. Five of the seven mutants have been tested in this manner and all show a striking effect in the displacement of the phenotype of $A^{br}\ A^d$ plants in a direction away from wild type as compared with sib $A^{br}\ a$ plants. The tests of the dosage effects of the A^d mutants require an additional generation and these studies are not completed. However, on the ears of the selfed $A^d\ a$ plants pale endosperms of three types occur, having one, two and three doses of the A^d gene. The phenotypes of the pale seeds on these ears are quite uniform suggesting that the A^d mutants show no dosage effect. More critical evidence on this point is being sought at the present time from studies involving comparisons in which a deficiency, instead of *a* is em-

ployed. These tests indicate that the A^d mutants which were obtained in the present experiment from $A^b\ a$ individuals are indistinguishable from those which have a known origin from $A^b\ A^b$ plants. It may be concluded that the former also are derivatives of A^b.

Since the heterozygous egg parents of the original cross were $A^b/lg\ a\ et$ and the pollen parents were $lg\ a\ et$, a linkage of lg or et with A^b in individuals from this cross is attributable to crossing over between A^b and the loci of the marker genes. Table 2 summarizes the information on recombination

TABLE 2

SUMMARY OF DATA ON CROSSING OVER IN THE *lg-a* (REGION I) AND *a-et* (REGION II) SEGMENTS FROM PROGENIES OF BACKCROSSES OF THE TYPE: $A^b/lg\ a\ et \times lg\ a\ et$*

	CROSSOVERS							
	REGION I		REGION II		REGIONS I AND II			
TOTALS	NUMBER	FREQUENCY	NUMBER	FREQUENCY	NUMBER	FREQUENCY	EXPECTED FREQUENCY	COINCIDENCE
17,298	5714	0.3303	2220	0.1283	405	0.0234	0.0424	0.55
(51 ears)		±0.0036		±0.0025		±0.0004		
22,706†	..		2946	0.1297				
(60 ears)				±0.0022				

* The data reported are from the two families which were involved in the original crosses and which produced the seven individuals carrying the mutant A^d gene.

† These more extensive data on crossing over in Region II include the 51 ears in the first row of the table along with additional progenies from the same families classified for *a* and *et* only.

for the *lg-a* and *a-et* regions obtained from progenies of the original crosses which produced the mutant individuals. Table 3 summarizes information

TABLE 3

SUMMARY OF THE LINKAGE INFORMATION FOR THE MUTANT A^d-BEARING INDIVIDUALS FROM THE CROSS: $A^b/lg\ a\ et \times lg\ a\ et$

	CONSTITUTION OF THE A^d-BEARING STRAND			
	NON-CROSSOVER	CROSSOVER TYPES		
MUTANT PLANT	TYPES	REGION I	REGION II	REGIONS I AND II
1A-1	...	.....	$Lg\ A^d\ et$	.....
8A-1	...	.....	" " "	.....
9A-1	...	.....	" " "	.....
14A-1	...	.....	" " "	.....
19A-1	...	.....	" " "	.....
78-1	...	$lg\ A^d\ Et$	.....	.....
78H-1	...	.....	.....	$lg\ A^d\ et$
Totals	0	1	5	1

as determined in progeny tests on the linkage phase of the etched endosperm and liguleless characters for the seven original plants carrying the mutant A^d gene. The interesting feature of these data is that in six of the seven cases the chromosome carrying the A^d gene emerged from the original cross combined with *et*.

Unless the change of A^b to A^d is in some way related to crossing over it would be expected that the frequency of crossing over in the *a-et* interval would be the same in sporocytes which carry A^d as in those which transmit A^b. On this basis, of the original mutant plants approximately 13% (table 2) A^d *et*/*a et* (crossover) and 87% A^d *Et*/*a et* (non-crossover) individuals are expected. The actual results were almost exactly the reverse of this; six of the seven individuals were crossover types for the *a-et* region. On the basis of 13% recombination and with no association between crossing over and mutation this number of mutants which are crossover types would be expected to occur among a total including approximately 40 mutants which are non-crossovers for the *a-et* region. There was only one of the latter.

The foregoing data suggest a relation between the mutation of A^b and crossing over in the *a-et* region. This interpretation receives further support from experiments conducted in 1948 on a larger scale. As before, marked heterozygotes carrying A^b were crossed with homozygous pollen parents of the constitution *lg a et*. In this case all of the pollen employed

TABLE 4

SUMMARY OF DATA ON CROSSING OVER IN THE *lg-a* AND *a-et* SEGMENTS FROM BACKCROSSES OF THE MARKED HETEROZYGOTES CARRYING A^b *

	REGION I		REGION II		REGIONS I AND II			
TOTAL	NUMBER	FREQUENCY	NUMBER	FREQUENCY	NUMBER	FREQUENCY	EXPECTED FREQUENCY	COINCIDENCE
5790	2068	0.3572 ±0.0063	804	0.1389 ±0.0045	184	0.0318 ±0.0023	0.0496	0.64

* These data from 34 ears in 23 families in 1948.

carried the *Dt* factor. Progeny tests of suspected mutant individuals from these crosses have not been made as yet. In the absence of these, however, all of the pale seeds with dotted endosperms have been selected and classified for normal versus etched phenotype. As indicated above by reason of their dotted phenotypes these seeds carry a mutant derivative of A^b and their selection on this basis is not subject to the error and prejudice which may be involved when classification is made solely on the basis of a character which may be quite variable such as the degree of pigmentation. Of the total of 34 pale, dotted, mutant seeds, 30 are crossover types for the *a-et* interval. Here as in the previous experiment there is an unexpected preponderance of the crossover class. In order to determine the normal rate of crossing over in the stocks employed in this experiment the total progenies from those ears on which mutant seeds occurred were classified. Since the mutant seeds occurred singly on the ears, a total of 34 ears is involved. The value obtained (table 4) for recombination in the *a-et* region (13.9%) agrees reasonably well with that obtained in the 1947 experiment.

The association of crossing over with mutation of A^b may be explained if it is assumed that there is some causal relation between the two. On one hypothesis it may be argued that occasionally a crossover in the *a-et* region results in an unequal exchange of chromatin at the locus of a gene which, like A^b, affects the pigmentation of plant and endosperm tissues. On this basis the pale phenotype of the "mutant" individuals would not be due to a change in the A^b gene but to a modification of chromatin somewhere between the *a* and *et* loci. Two types of evidence are against such an interpretation. (1) In all cases dots, resulting from the *Dt*-induced mutation of *a* to *A*, have been observed on the pale background of the mutant seeds or of pale seeds in their progenies. If the pale background of these seeds is owing to the action of a modifier gene, the phenotype of

TABLE 5

ACTUAL AND THEORETICAL FREQUENCIES OF CROSSOVERS AND NON-CROSSOVERS RELATIVE TO THE *a-et* INTERVAL FOR THE A^d-BEARING STRANDS OF THE FORTY-ONE MUTANT INDIVIDUALS*

BASIS ON WHICH FREQUENCIES ARE COMPUTED	CROSSOVERS	NON-CROSSOVERS	STANDARD ERROR
Observed	0.878	0.122	0.051
No relation between mutation of A^b and crossing over	0.132	0.868	0.053
Mutation of A^b conditioned by crossing over at that locus. No allowance for interference	0.868	0.132	0.053
Same as above but allowing for coincidence of 0.5	0.934	0.066	0.039

* Actual values are based on the total of 41 mutants, 36 of which were crossover types. Theoretical values are based on the average rate of crossing over for this region (13.2%) taken from tables 2 and 4.

cells included in the dots should be modified to pale also. (2) If the pale phenotype of mutant seeds is held due to a modifier it is clear that the latter must be dominant since the cells of these endosperms carry a normal chromosome from the pollen parent. In this case the selfed or suitably crossed progenies of mutant individuals should produce some offspring which are homozygous recessive for the modifier condition and carry A^b; these would appear as recovered cases having purple endosperms and purple plant phenotypes. No such recoveries have been noted in the seven cases for which progenies are presently available.

These two types of evidence not only rule out the possibility of a modifier effect in the occurrence of the mutant seeds but indicate further that the pale effect of the mutation must be due to a change in the A^b gene itself. On any other basis the mutations of *a* (for which these seeds are heterozygous) to *A* through the action of *Dt* would not be recognizable as dots on these seeds; nor would the absence of purple seeds and plants in the

progenies of the mutant individuals be expected. Since it is necessary to relate the high frequency of crossing over to this change the simplest and most direct hypothesis is that these mutations of A^b to A^d are a result of crossing over within the locus of A^b.

In the cases of 5 of the total of 41 mutants from both experiments the A^d-bearing strand delivered to the egg was seemingly a non-crossover type for the *a-et* interval. In view of the relatively great length of this segment (13.2 units as an average in these experiments) it is possible to explain these apparent non-crossover types as due to double exchanges, one occurring at the locus of A^b, giving rise to the mutation, the other occurring somewhere between this locus and that of *et*, thus reconstituting the parental combination. The data of table 5 are pertinent to this argument. Here are presented the actual frequencies of crossover and apparent non-crossover strands carrying the mutant A^d gene alone with frequencies expected on three theoretical bases. The greatest discrepancy is between observed values and those expected on the basis of no relation between mutation and crossing over (second row). In the third row of the table are given the frequencies expected on the hypothesis that the mutation of A^b is caused by a crossover at that locus. On this basis the non-crossover class results from double exchanges. Here there is good agreement between the theoretical and experimental frequencies. The value 0.122 for apparent non-crossovers among the 41 mutant cases is close to the average value of 0.132 for crossing over in the *a-et* region (from tables 2 and 4). However, in the computation of the theoretical frequencies in the third row of table 5 no allowance has been made for interference. The frequency of 0.122 for non-crossovers (reasoned as cases of double exchange) among the 41 mutant cases corresponds to a coincidence of 0.9. This exceeds the coincidence value of 0.6 corresponding to the *lg-a* and *a-et* regions (tables 2 and 4). In view of the small number of mutant cases it is not clear whether the excess of apparent non-crossover types among them is due to errors in sampling or to the occasional occurrence of mutations of A^b which are not associated with crossing over. If mutations of the latter type occur a large proportion of these would fall in the non-crossover class thus tending to increase unduly the coincidence value. There is at present no basis for estimating the effect of a crossover at the A^b locus on the frequency of coincidental exchange in the *a-et* region but it is reasonable to expect that the value for coincidence in this case would fall below 0.6. In the last row of table 5 are given the theoretical frequencies of crossovers and apparent non-crossovers associated with an arbitrarily chosen coincidence of 0.5; from the corresponding standard error for $N = 41$ it is clear that the actual frequencies could well have been chosen from such a population.

Confirmatory evidence of the association of mutation of A^b and crossing

over is being sought from independent experiments using plants in which the A^b segment of the third chromosome either has no homologous region with which to synapse (in hemizygous plants) or, in cases where A^b is carried in a duplication, competes at a disadvantage for synapsis. Since these experiments are designed to eliminate or suppress those A^b mutations due to crossing over they may be expected to establish also whether mutations of A^b to A^d which are not associated with crossing over can occur.

In the foregoing presentation occasional reference has been made to the "A^b locus." There is strong argument from the present evidence for viewing the "A^b locus" as composed of at least two, physically distinct units which function coöperatively in the determination of the A^b effect. If these are designated alpha and beta, the latter being more distal, the A^d mutants may be described as having alpha and lacking beta since the strands carrying A^d in these experiments are predominantly of the non-parental class for *et*, the more distal marker gene. This consideration poses several interesting problems whose analysis may be viewed as critical tests of the hypothesis. (1) Since on this basis A^b is composed in part of the A^d or alpha component the action of A^b may be interpretable, at least in part, in terms of effects which, among those of the A alleles, are distinctive for A^d. As reported elsewhere[5] these include the determination of brown pericarp which is dominant to the red effect of A, the absence of a cumulative effect with increasing doses, and a striking tendency to reduce rather than enhance the type effect in combinations with various A alleles. Of the alleles associated with purple aleurone and plant phenotypes, only A^b has a brown pericarp effect which is dominant; it is reasonable to infer that this action may be assigned to A^d which has been argued as a component of A^b. It is not known whether A^b shares the dosage and competitive effects of A^d but this information is technically more difficult to obtain for A^b than for A^d because of the closer approach of the former to wild type. (2) If, as on the present scheme, the A^d mutants represent the alpha component which has been separated from the beta constituent by crossing over between the two, it is expected that another class of mutants corresponding to the beta component carried on the reciprocal strands involved in such exchanges should occur with equal frequency. While these have not been isolated this is no argument against their occurrence. A^b is associated with purple aleurone and plant while what has been argued as its alpha component, A^d, is distinctly intermediate in effect. From this it seems likely that the beta component produces a purple phenotype and if so mutants of this type would be indistinguishable from seeds carrying the unchanged A^b. However, on the expectation that the beta component produces a pericarp color distinguishable from A^b, experiments are being conducted to determine whether A^b gives rise to mutants of this type. These would have escaped detection in the experiments reported here

since the P factor which has a complementary effect in the pigmentation of this tissue was absent. (3) Since the A^d mutants are argued to represent a more proximal component of A^b considerable interest attaches to whether these mutants are similar in their action. If the alpha component is itself compound in nature it might be predicted that the A^d mutants in turn are not a homogeneous lot. Comparisons in constant genetic backgrounds of the mutant alleles of the present experiments are not available now; from observations of related but non-sib mutant plants the impression is given that these alleles will prove to be quite similar in effect. Nevertheless, the possibility of the component nature of alpha remains a particularly inviting prospect. Studies[5] on the action of A^b and several mutants of the A^d type indicate that their action cannot be explained on the basis of a simple relation between gene and reaction. Provisional schemes were offered to account for the exceptional behavior of these alleles. It was suggested (*a*) that the determinants corresponding to these genes function as units but that the enzyme corresponding in each case might participate in more than a single reaction, or alternatively (*b*) that a simple relation biochemically between gene and enzyme might be defended on the assumption that each of these alleles is compound in the sense of being constituted by more than one physically distinct determinant. The evidence reported here suggests that the antimorphic action of A^b resides in such a component nature. It is possible that the A^d mutants likewise owe their unusual behavior to a similar cause but proof of this requires direct evidence of the compound nature of what has been labeled here the alpha component. (4) Is the crossover which gives rise to the mutants preceded by unequal synapsis? Since the mutations occurred in plants heterozygous for a it follows that any statement about such an arrangement must be based on some understanding of the physical constitution of this allele and on this point there is no information. There is hence no basis from these experiments to argue that the change is related to unequal synapsis or even that there is any final inequality of exchange. However the mutants which Stadler[6] obtained and which are similar in effect to the A^d mutants of the present experiments occurred in homozygous A^b individuals. It is not known whether these mutations were associated with crossing over, but if so, there is a basis for the argument that unequal synapsis is involved. Making use of more efficient markers which have become available recently, the relation between crossing over and mutation in $A^b A^b$ plants is being investigated.

It is premature to discuss the relation of the present case to others which have been more extensively studied.[1-3] Nevertheless it is a rather inviting prospect, in analogy with the cases of Bar, lozenge, Star-asteroid and others[10] in *Drosophila* and with that in *Gossypium*,[11, 12] that A^b represents repeated loci and by further analogy with several of these cases has com-

ponents which are derived by duplication from a common ancestral locus and which have since become modified. No less attractive is the possibility that the divergent action of certain of the *A* alleles may find an explanation in terms of their component nature.

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*ABERRANT HETEROZYGOTES IN ESCHERICHIA COLI**

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A mechanism of genetic recombination has been indicated in experiments on *Escherichia coli*, strain K-12.[1] A synthetic agar medium was used as a selective sieve to isolate occasional prototroph recombinants which appear in mixed cultures of complementary biochemical mutants. Later, additional genetic factors were introduced, including fermentation and virus-resistance mutations, and these factors were found to segregate in characteristic ratios, suggesting linkage. All the cells in a given prototroph colony showed the same combination of characters and were stable on further cultivation. Therefore, it was inferred that segregation had occurred before the initiation of the colony, and that the postulated zygote had a very short life, probably a single cell generation. The life cycle would resemble the ascomycete's, in which haploid nuclei fuse to form a transient diploid zygote which undergoes meiosis without any intervening mitoses.

Exceptions to this rule have now been found in the form of unstable prototroph cultures which continually segregate out various recombination types so as to suggest that they are heterozygous and diploid. But

abnormalities in the segregation ratios suggest some sort of chromosomal aberration whose nature has not yet been proved.

Material and Methods.—The cultural and crossing techniques used earlier have generally been followed. Crosses were also made by spreading mixtures of cells on the surface of synthetic medium[2] to which carbohydrate and an eosin-methylene blue indicator are added. This medium, EMS-, classifies prototrophs as soon as they appear for fermentation of the sugar used. Other media used include EMB- agar, in which the indicator is incorporated in a sugar peptone base. The genetic factors involved in this study are summarized in table 1.

TABLE 1

SUMMARY OF MUTANTS

LOCUS	"TYPE" ALLELE	MUTANT ALLELE	CHARACTER OF MUTANT
B	+	−	Needs biotin for growth
M	+	−	Needs methionine
T	+	−	Needs threonine
L	+	−	Needs leucine
B_1	+	−	Needs thiamin
Lac	+	−	Unable to ferment lactose
Mal	+	−	Unable to ferment maltose
Gal	+	−	Unable to ferment galactose
Ara	+	−	Unable to ferment L-arabinose
Xyl	+	−	Unable to ferment D-xylose
Mtl	+	−	Unable to ferment mannitol
V_1	*s*	*r*	Resistant to phages T1, T5
V_{1c}	*s*	*r*	Resistant to T1; sensitive to T5
"*Het*"	..	..	Persistent heterozygote factor

Crosses Involving "Het."—The first unstable prototroph to be discovered, "*H-1*," arose in a cross to test the allelism of the virus resistance factors V_1 and V_{1c}. The parents were B−M−Lac+ $V_{1c}r$ and T−L−B_1−Lac− V_1r. Among 200 prototrophs picked and tested on synthetic agar for sensitivity to T1, only one was an apparent crossover showing a full sensitive reaction. However, when this unique culture was streaked out on EMB−lactose, it gave rise not to typical dark Lac+ or light Lac− colonies, but to highly variegated colonies with intermingled sectors of light and dark cells. The variegated reaction will be referred to as Lac^v.

When Lac^v colonies were streaked out again on EMB−lactose, pure Lac−, pure Lac+ and a few Lac^v colonies were seen. But when the pure − and + colonies were inoculated on EMS−lactose they failed to grow, showing that they were nutritionally deficient. Furthermore, although they had been derived from a virus-sensitive culture, most of the purified "segregants" were resistant to T1 when tested on EMB−lactose. On the other hand, Lac^v colonies gave rise to normal appearing Lac+ on

EMS − lactose, which, as before were sensitive to T1. Taken back to EMB − lactose, they showed the Lac^v result of splitting off pure − and + types. When single colonies were streaked out in series for 15 transfers on EMS − lactose, they retained their normal Lac+ appearance, but throughout the experiment they continued to segregate when brought on EMB − lactose, which is a nutritionally "complete" medium. To summarize, *H-1* was an unstable prototroph culture which was apparently Lac+ and T1-sensitive so long as it was maintained on a synthetic medium, but which segregated nutritionally deficient, Lac− and T1-resistants on a "complete" medium.

At this point, three alternatives had to be distinguished. The segregating entities might be (*a*) associations of intact cells of the original parental mutants collaborating by syntrophism to allow growth on synthetic medium; or (*b*) heterokaryons comprising intact nuclei of the parents in the same cell; or finally (*c*) diploid heterozygotes. The phage sensitivity of *H-1*, contrasted to the resistance of the parents, render (*a*) unlikely, but the segregation might still be from a heterokaryon or a heterozygote. To test these alternatives, it was thought that a heterokaryon should split out each of the two parental genotypes, but no additional combinations. On the other hand, segregation from a heterozygote should sometimes be accompanied by crossing over, and the formation of new combinations of the parental characters.

One hundred thirty-five Lac^v colonies were separately streaked out on EMB − lactose, and a single pure Lac+ and Lac− segregant isolated from each of the 135 streakings, to insure that each of the cultures tested was an independent segregant. Among the 135 Lac− tested, there were 121 T5^r (parentals) and 14 T5^s (exchanges); the Lac+ included 133 T5^s (parentals) and 2 T5^r (exchanges). Therefore, out of 270 tests there were 16, or 6%, new combinations. Therefore, crossing over occurs during the segregation of *H-1*. This is compatible only with the hypothesis of segregation from a heterozygote.

The following remarks should not obscure this major conclusion. The amount of exchange in the Lac− and Lac+ series is not the same ($\chi^2_1 = 9$). The crossovers in the two series came from different Lac^v colonies, but reciprocality cannot be demanded if many different segregations occur during the growth of a single Lac^v colony. Even the higher value of $^{14}/_{135}$ or 10% for exchanges between Lac and V_1 in the Lac− series is lower than the 38% derived in previous studies on prototroph recombinants. Finally, it was very obvious that the Lac− segregants far outnumbered the Lac+. If anything, selective pressures on EMB − lactose seem to favor Lac+, so this finding was most unexpected.

Owing to technical difficulties, tests on nutritional segregations have been less extensive. It proved to be especially difficult to score for the

biotin requirement in the presence of amino acids, probably due to partial replacement. The classification of the thiamin requirement (B_1-) also requires fastidious attention to clean chemicals and glassware. Most of the Lac+T5^s segregants are the parental B−M−; Lac−T5^r are usually T−L−B_1→. However, prototrophs and the multiple mutant combinations M−T−L− and M−T−L−B_1− have been encountered. These combinations could not be obtained by previous methods, which necessarily isolated prototrophs.

Unstable heterozygotes like *II-1* had not been encountered before, although many hundreds of Lac+ prototrophs from crosses heterozygous for Lac had been streaked out on EMB−lactose. The persistence of *II-1* as a diploid might be a non-genetic accident or perhaps an effect of a spontaneous life cycle mutation. If so, it would be possible to set up crosses to yield diploids heterozygous for additional markers.

Three segregants from *II-1* have been used in crosses to test for the production of persistent heterozygotes. (*A*) (W-466) was B−M−$Lac_1-V_1^s$; (*B*) (W-477) T−L−B_1−Lac_1−V_1^r and (*C*) (W-478) B−M−Lac+V_1^s. These cultures were crossed on EMS−lactose with appropriate, "standard" complementary stocks (B−M− or T−L−B_1−; Lac− or Lac+) of independent origin. Lac+ prototrophy were streaked out on EMB−lactose, and Lac^r colonies looked for. In each of these crosses, about 5% of the Lac+ prototrophs were heterozygous. Crosses of $A \times C$ gave the same result. If "*Het*" is located on a chromosome, it is effective whether present in one or both parents.

In further studies still in progress, *C* has been crossed with multiple fermentation mutant stocks, T−L−B_1−Lac_1−Mal−Gal−Ara−Xyl−, and some also Mtl−. Such stocks were developed by irradiations on EMB− media, in sequence. As before, about 5 to 10% of the + prototrophs isolated from EMS lactose or xylose plates are heterozygous. However, these cultures are not uniformly heterozygous for all the factors in which the parents differed. It is especially notable that in over a hundred heterozygotes obtained in this way, Mal has never been heterozygous. Usually, the cultures are pure Mal−, sometimes Mal+. Xyl^r cultures are equally often Lac^r or pure for Lac, as well as the converse.

It remains to be decided whether the "pure" characters are homozygous or hemizygous. The Mal locus which, as mentioned, has always been "pure" is probably hemizygous, as shown by reversion studies. If the cultures were homozygous, Mal− would be twice represented, i.e., Mal−/Mal−. Reverse mutations of one of these genes should lead to heterozygosity at the locus. Preliminary tests on reversions for Mal in these stocks have invariably given pure Mal+ cultures, although they still segregated for other factors. This suggests that Mal is represented only once, that it is hemizygous, and, therefore, that there is deficiency for a

region including this locus. This tentative conclusion is in accord with the aberrant segregation of Lac (excess of Lac−) observed in *H-1*. The hypothetical deficiency would be lethal in a haploid, and lead to the loss of segregants carrying the alleles linked to it.

Some quantitative studies of segregation and crossing over have been carried out on these complex heterozygotes. *H-72* segregates both for lactose and xylose fermentation. Segregation was permitted to occur "enmasse" in heavy cultures in nutrient broth, the cultures diluted and plated on EMB− xylose and EMB−lactose. Of 895 colonies on EMB−xylose, 19 were still Xyl*v*; 815 Xyl− and 61 Xyl+. The Xyl+ were all Lac−. The segregation ratio is 815−:61+ or more than 13:1. Similarly, of 753 colonies on EMB−lactose, 23 were still Lac*v*; 654 Lac− and 76 Lac+ (all of which were Xyl−). Thus, no Lac+Xyl+ segregants were observed in this sample. The other classes are computed to be Lac−Xyl− 83%, Lac−Xyl+ 7% and Lac+Xyl− 10%. These figures might be accounted for by mapping:

Xyl+ *7.7* Lethal *11.2* Lac+/Xyl− . . . Lac−.

To justify these conclusions, independent mapping of Xyl, Mal, Lac will be necessary.

Four hundred ten segregants were tested both on EMB−lactose and on EMB−xylose. Segregation was always complete; i.e., no cultures were found which had segregated for one factor, and not for the other. This eliminates interpretations based on new segmental losses, or on some forms of autogamy.

It may be argued that the unequal segregations are due to selection. This factor cannot be excluded, but the dominant segregant differs from diploid to diploid. That is to say, other heterozygotes have been isolated from parallel crosses which gave an excess of Lac+ rather than Lac− segregants, or Xyl+ more than Xyl− or both. It seems more likely that the ratios observed are based upon the genetic constitution of the heterozygote.

The Lac+ character of Lac_1 heterozygotes shows that Lac_1+ is dominant to Lac_1−. A number of other loci have been identified, mutations at which lead to the inability to ferment lactose. At two more of these at least, the + allele is dominant. Cultures carrying Lac_2− or Lac_4− were crossed to "*Hfr*" stocks carrying Lac_1−. A large proportion of the Lac+ prototrophs seen on EMS−lactose turned out to segregate for lactose fermentation. Their variegation, however, was generally periclinal rather than sectorial, with dark, Lac+ centers and light, Lac − margins with only occasional streaks of Lac+. This difference is to be expected, since each of the chromosomes of the diploid carries a Lac− mutation. Therefore, only those segregants in which there has been a crossover

between the two Lac loci, bringing the + alleles into coupling phase will be lactose positive. That these cultures are segregating for two Lac− factors has been confirmed by physiological and genetic tests on the segregants.

Heterozygotes from Standard Crosses.—All the heterozygotes so far referred to are the issue of crosses involving the hypothetical "*Het*" factor derived from *H-1*. In these crosses, several per cent of the prototrophs are demonstrably heterozygous. Previous and current controls showed that, if they occurred at all in "normal" crosses, they must be very much rarer. In order to make a more thorough test, advantage was taken of the very close linkage (less than 1% recombination) which has been observed between the Lac_1 and Lac_4 loci mentioned above. B−M−Lac_1−Lac_4+ was crossed with T−L−B_1−Lac_1+Lac_4−. Much less than 1% of the prototrophs of this cross on EMS−lactose are Lac+. It was reasoned that a Lac+ prototroph might represent either a very rare crossover, or a diploid in which the + factors were carried on opposite chromosomes. Because most haploid recombinants, being Lac−, can be set aside by inspection, this is a fairly efficient way of screening for rare diploids. In this way, heterozygous diploids were also obtained from normal stocks, not carrying "*Het*," but they constitute only about 0.1% of the prototrophs. These prototrophs are generally similar to the previous ones, except that they are somewhat more stable. It has not been established whether new "*Het*" mutations have occurred here. When these diploids are singly heterozygous for various sugar fermentation factors, they also show non-random segregations, but these mutations are not the same as those in the "*Het*" series, so that a direct comparison is not yet possible.

Discussion.—It is not clear what relationship there is between the exceptional persistence of these diploids, and the abnormalities in their segregation. There may be some direct connection between their heteroploidy, i.e., deficiency for the Mal region, and the prolongation of the diplophase. But the very disturbing possibility has not been discounted that these heterozygotes merely reveal a situation which also operates in the formation of haploid prototrophs from transient zygotes. If this is so, the linkage map of *E. coli* K-12 will have to be systematically re-examined, with the use of several unrelated sets of stocks. In any case, the discrepancies affect only the details of chromosome behavior. These heterozygotes, on the other hand, have provided an unexpected confirmation of the sexual basis of genetic recombination in this bacterium.

Although the heteroploidy is somewhat of a limitation, heterozygote formation is a very helpful tool in genetic analysis. Since all types of recombinants, not only prototrophs, can be recovered, it is feasible to extend pedigrees to several generations, and interesting combinations of factors can be put together in a form allowing their use in crosses. For

example, a Lac_1- mutation occurring in a $T-L-B_1-$ stock has been transferred to a combination with $B-M-$, allowing it to be compared genetically in crosses with other Lac− mutants induced in $T-L-B_1-$ material.

The experimental production and maintenance of diploids raises questions of dominance and dosage effects. It has already been noted that the normal alleles of several biochemical mutations are dominant, although quantitative comparisons of enzyme competence in heterozygotes, haploids and homozygotes remain to be carried out. The dominance of the type sensitive alleles of genes for phage resistance supports, at least in part, a segregation interpretation for the delayed effects noted by Demerec and Latarjet in induced resistance mutations.[3] On the other hand, for the study of phenotypic lag in bacteria,[4] segregation provides a far larger and more reproducible source of material than mutation.

In respect to lactose fermentation, such a lag cannot last more than a few cell generations in this material, in view of the appearance of numerous Lac− sectors in Lac+ colonies on EMB−lactose agar. The masking effects noted[5] with yeast asci segregating in the presence of the substrate have thus not beeen found here.

Cytological and single cell studies on these strains are in progress.

Summary.—1. Unstable prototrophs have been isolated from certain crosses in *E. coli* K-12, and characterized as segregating heterozygous diploids.

2. The capacity to produce appreciable numbers of persistent heterozygous diploids is inherited.

3. The segregation of various mutant factors is strongly biased, possibly due to a recessive lethal deletion including a locus affecting maltose fermentation.

4. In the heterozygotes, the type + alleles of factors controlling several fermentations and nutritional requirements are dominant. Sensitivity to bacteriophage T1 is dominant to resistance.

* Paper No. 391 from the Department of Genetics, College of Agriculture, University of Wisconsin. This work has been aided by a grant from the Wisconsin Alumni Research Foundation. The author is indebted to Professor James F. Crow for his stimulating discussions.

[1] Tatum, E. L., and Lederberg, J., *J. Bact.*, **53**, 673–684 (1947). Lederberg, J., *Genetics*, **32**, 505–525 (1947).

[2] The composition of EMS-, in grams per liter, is: Sodium Succinate 5; $(NH_4)_2SO_4$ 5; NaCl 1; $MgSO_4$ 1; K_2HPO_4 2; Agar 15; Methylene Blue Hydrochloride 0.065; Eosin Y 0.4. This formula is conveniently stored as a dry mixture of the powdered components.

[3] Demerec, M., and Latarjet, R., *Cold Spring Harbor Symposia Quant. Biol.*, **11**, 38–50 (1946).

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THE IN VITRO SYNTHESIS OF PANTOTHENIC ACID BY PANTOTHENICLESS AND WILD TYPE NEUROSPORA

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It has been demonstrated by Wagner and Guirard[1] that the resting mycelium of wild type *Neurospora* is able to synthesize pantothenic acid in the presence of β-alanine and pantoyl lactone (DL-α-hydroxy-β,β-dimethyl-γ-butyrolactone). The *in vivo* synthesis of pantothenate from these precursors cannot be performed, however, by the pantothenicless mutant 5531. If the wild type mycelium is treated with acetone to kill the cells and then dried with acetone, the resultant preparation can also be shown to possess an active enzyme system capable of carrying out the above synthesis *in vitro*.[1]

This present communication is concerned with an extension of the investigation of *in vitro* synthesis of pantothenate by *Neurospora*. Three strains, Emerson wild type 5256A, and two pantothenicless mutants, 5531A and 34556A, were used. Both mutants appear to be due to a change at the same gene locus. The two differ in that 5531 was induced by x-rays and 34556 by ultra-violet light.[2]

Experimental.—Activity of Intact and Water Extracted, Dried Mycelium: Fresh Mycelium of 5256, 34556, and 5531 was treated with acetone according to the method previously described.[1] The resultant preparations are described in what follows as intact, acetone-dried mycelium. The activity of these preparations with respect to the production of pantothenic acid from the precursors, β-alanine and pantoyl lactone was tested at three different temperatures. In each case the reaction mixture consisted of 50 mg. of acetone-dried material/flask suspended in 25 ml. of 0.04 *M* potassium phosphate buffer at pH 6.5. In the case of those flasks to which precursors were added, an initial concentration of 0.004 *M* of each precursor was used. The flasks and contents were sterilized by autoclaving and cooled before the addition of the acetone preparations. Incubation was carried on for twenty-four hours with constant shaking at 22°, 27° and 35°C. The flasks were then steamed, the solids filtered out and the filtrate assayed for pantothenate with *Lactobacillus arabinosus* by the method of Skeggs and Wright.[3] The results are presented in table 1, as μg. pantothenate produced/50 mg. mycelium/twenty-four hours, both in the presence and absence of the two precursors.

Ten grams of each of the intact, acetone-dried preparations were washed in 1000 ml. of distilled water for several hours with constant shaking and then filtered. The residues (R) were dried with acetone and weighed.

In the case of each strain the residue was found to have lost ca. 50% of the original weight of the unextracted preparation. The filtrates were each reduced *in vacuo* to 200 ml. The water-insoluble residues (R) were tested for activity in the same way that the intact preparations were tested using 50 mg. of R per flask. The concentrated filtrates (S) were sterilized by Seitz filtration and tested for activity at 35° with and without the corresponding residues. An amount of S (1 ml.) equivalent to 50 mg. of intact, acetone mycelium was added to the flasks. Table 2 presents the results of these tests.

Activity of the Concentrated Water Extract: The concentrated filtrate (S) from each strain was added in several different ml. concentrations to a set of flasks containing the precursors and 50 mg. of the respective residues.

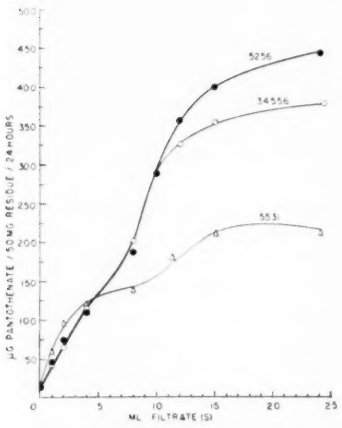

FIGURE 1

The effect of added excess filtrate (S) on the production of pantothenate.

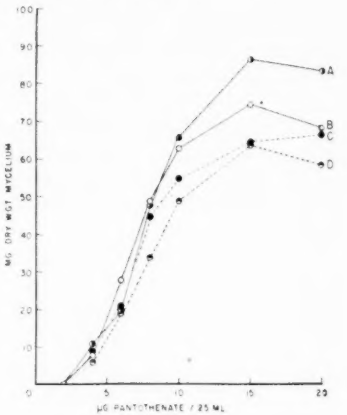

FIGURE 2

The effect of the precursors on the growth of the pantothenicless mutants. A, 5531 plus precursors; B, 5531 without precursors; C, 34556 without precursors, D, 34556 plus precursors.

Incubation was at 35° for twenty-four hours. The results are described by figure 1.

The Effect of the Precursors on the Growth of the Mutants: The mutants 34556 and 5531 were grown for four days at 35° and pH 6.5 in the presence of 0.004 *M* β-alanine and pantoyl lactone, and various concentrations of Ca pantothenate. A control series without the precursors, but with Ca pantothenate, was tested simultaneously. The results of this experiment are given in figure 2.

Growth of the Mutants on Pantothenate Synthesized in Vitro: The panto-

thenicless mutants were inoculated into media containing various concentrations of pantothenate synthesized by the acetone preparations of these same strains which had been previously assayed with *L. arabinosus*. The pH of the medium was at 6.5, the temperature, 35°, and the incubation period, four days. Controls were run simultaneously with Ca pantothenate. Figure 3 shows the growth response of 5531 to pantothenate synthesized by 5531 *in vitro*, and figure 4 the response of 34556 to its corresponding source of pantothenate. The data from the corresponding controls are included in each case for comparison.

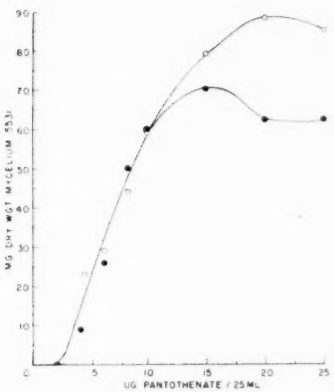

FIGURE 3

Growth of 5531 on Pantothenate synthesized *in vitro* by 5531.

○ pantothenate synthesized by 5513
● Ca pantothenate control

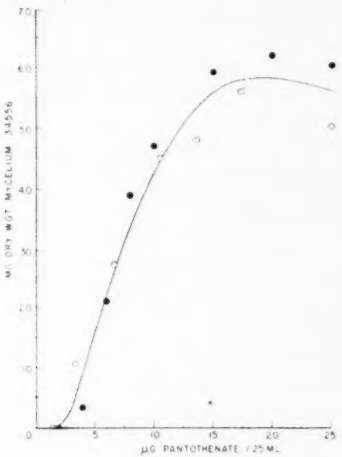

FIGURE 4

Growth of 34556 on pantothenate synthesized *in vitro* by 34556.

○ pantothenate synthesized by 34556
● Ca pantothenate control

Discussion.—The data presented in tables 1 and 2 demonstrate that the pantothenicless mutants, 5531 and 34556, as well as the wild type *Neurospora*, possess an enzyme system capable of carrying out the *in vitro* synthesis of pantothenic acid from β-alanine and pantoyl lactone. There is a difference indicated in the system possessed by the mutants when compared to the wild type with regard to the effect of temperature, but the enzyme is active to the same degree in all three strains at 35°. The enzyme in each strain consists of at least two parts, a water-insoluble residue (R) and a water-soluble portion (S) which is inactive alone, but which increases the activity of R when combined with it.

The data in figure 1 when combined with the data in tables 1 and 2 show

that the concentration of S is limiting, with the concentration of precursor used, in the synthesis of pantothenate by the intact and washed acetone preparations. The amount of pantothenate synthesized in the presence of excess S, as shown in figure 1, leaves no doubt that there is present in all three strains a mechanism active in this synthesis. This considerable increase in pantothenate production cannot be accounted for by the pantothenate present in S, which is 0.31, 0.48 and 0.65 μg./ml. for 5256 (S), 5531 (S) and 34556 (S), respectively.

TABLE 1

THE ACTIVITY OF INTACT, ACETONE-DRIED MYCELIUM

ACETONE PREPARATION	22°		27°		35°	
	O	P	O	P	O	P
5256	4.0	12.5	2.8	55.0	2.3	48.0
5531	0.51	0.55	1.8	13.0	1.5	47.0
34556	4.2	8.3	1.5	9.5	1.5	45.0

μg. pantothenate produced/50 mg. intact, acetone-dried mycelium/twenty-four hours by 5256 (wild type) and two pantothenicless strains, 5531 and 34556, at three different temperatures. O designates absence and P presence of both pantoyl lactone and β-alanine.

TABLE 2

THE ACTIVITY OF WATER-SOLUBLE AND WATER-INSOLUBLE FRACTIONS

REACTION MIXTURE	5256	5531	34556
Residue alone	0.43	0.55	0.90
Residue + precursors	12.0	13.0	12.0
Filtrate + precursors	0.42	0.65	0.65
Residue + filtrate + precursors	48.0	78.0	68.0

μg. pantothenate produced/50 mg. residue/twenty-four hours. The amount of filtrate added was equivalent to 50 mg. of intact acetone dried mycelium. Temperature at 35°C.

The water-soluble factors appear to be identical in activity for the three strains, since they can be substituted for one another with results comparable to those given in figure 1. In addition the factor or factors are dialyzable and heat stable while the water-insoluble fraction is not. Further work has been done on the coenzyme indicated, and these results will be recorded elsewhere. It is evident that 34556, 5531 and 5256 each have the same or a similar coenzyme involved in the production of pantothenic acid from β-alanine and pantoyl lactone.

In all of the experiments discussed above the production of pantothenate was determined by assaying with *L. arabinosus*. It was necessary, therefore, to establish the activity of the pantothenate for the mutants. Since the reaction mixtures containing pantothenate also had uncombined pantoyl lactone and β-alanine present, the effect of these compounds on the growth of the mutants was determined as shown in figure 2. It can be seen from these data that no growth occurs in the absence of panto-

thenate with the precursors present, and there is no increase in growth over the controls in the presence of the precursors and limiting amounts of pantothenate. There does seem to be a slight but consistent beneficial effect of the precursors on the growth of 5531 at optimal concentrations of pantothenate.

In view of the inactivity of the precursors, the data in Figures 3 and 4 definitely establish the activity of the pantothenate synthesized by 5531 and 34556 *in vitro* for these mutants. The results reported here were obtained by using pantothenate synthesized in the presence of excess coenzyme. Similar results were obtained by using the pantothenate synthesized by intact, acetone-dried mycelium, and also with pantothenate synthesized by wild type.

These findings are of interest in connection with the current hypotheses dealing with the postulated gene enzyme relationship. According to one of these hypotheses[4] 5531 and 34556 are mutant because they are incapable of synthesizing their own pantothenic acid due to a blocking of the reaction coupling β-alanine and pantoyl lactone. The block may be caused by the absence of the necessary enzyme or coenzyme or by inhibition. This interpretation is supported by the fact that (1) wild type is stimulated to produce pantothenic acid by the presence of these two compounds,[1] (2) by the demonstration that these compounds together do not support growth of the mutants, and (3) by the report that these precursors accumulate in the medium when the mutant 5531 is grown in the presence of pantothenate.[5] (Strain 34556 has not as yet been tested for accumulation of precursors.) The data presented in this report rule out the enzyme absence hypothesis for these mutants, assuming that we are correct in concluding that it is this particular step in the synthesis of pantothenate which is involved. It is then necessary to conclude that the mutants possess a mechanism which is active *in vivo* in preventing this enzyme system from operating in pantothenate synthesis.

Summary.—The pantothenicless mutants 5531 and 34556 of *Neurospora* possess, like wild type, an enzyme system for the synthesis of pantothenic acid from β-alanine and pantoyl lactone *in vitro*.

* A portion of this work was done while the author was a research fellow at the California Institute of Technology during the summer of 1948.

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BOUNDS FOR THE SOLUTIONS OF A SECOND-ORDER LINEAR DIFFERENTIAL EQUATION

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In this note we consider self-adjoint differential equations of the form

$$(r(x)y')' + p(x)y = 0, \tag{1}$$

where r, p and $(rp)'$ are continuous and r and p are positive on I: $a \leq x < \infty$.

THEOREM. *If $(rp)' \geq 0$ on I, every solution $y(x)$ of (1) is bounded on I. If $(rp)' \leq 0$ on I, $|y'(x)| \leq M/r(x)$ on I.*

It is easy to verify that if $y(x)$ is a solution of (1),

$$(ry')^2 + rpy^2 = c^2 + \int_a^x (rp)'y^2dx, \tag{2}$$

where c^2 is a positive constant. Suppose the maximum M of $|y(x)|$ on $[a, x]$ is assumed at $x = \xi$. Then

$$r(\xi)y'(\xi)^2 + r(\xi)p(\xi)M^2 = c^2 + \int_a^\xi (rp)'y^2dx,$$

and

$$r(\xi)p(\xi)M^2 \leq c^2 + M^2 \int_a^\xi (rp)'dx.$$

It follows that $M^2 \leq c^2/r(a)p(a)$. Since $c^2/r(a)p(a)$ is independent of x, the first statement in the theorem is proved. The second statement in the theorem is an obvious consequence of (2). One notes incidentally that if $(rp)' \leq 0$ on I, $rpy^2 \leq M$.

In the corollaries which follow the theorem is applied to determine bounds for the solutions of a differential equation in so-called *normal* form of (1):

$$y_0'' + p_0(x)y_0 = 0, \tag{3}$$

where we assume $p_0(x)$ to be class C' on I. It will be observed that the method employed is also available for equations not in this form.

COROLLARY 1. *If $R(x)$ is a positive function of class C''' on I such that*

$$rp = R^3(R'' + p_0R) > 0 \tag{4}$$

and $(rp)' \geq 0$ on I, every solution $y_0(x)$ of (3) satisfies the condition

$$|y_0(x)| \leq MR(x).$$

To prove the corollary it is sufficient to note that the transformation $y_0 = r^{1/2}y(r^{1/2} = R)$ carries (3) into (1), where p is defined by (4).

"Bestness" of the corollary's conditions follows from the readily verifiable fact that the general solution of the differential equation $R^3(R'' + p_0R) = 1$ can be given the form $R^2 = ay_1^2 + by_2^2 + 2cy_1y_2$ $(ab = \delta^{-2} + c^2)$, where δ is the Wronskian of the solutions y_1 and y_2 of (3).

A frequently useful consequence of Corollary 1 may be obtained by writing $x^2p_0 = q$. We have then the following result.

COROLLARY 2. *If there exists a number* α *such that* $\pi = x^{4\alpha-2}(q - \alpha + \alpha^2) > 0$ *and* $\pi' \geq 0$ *on* I, *a solution* $y_0(x)$ *of* (3) *has the property that* $|y_0(x)| \leq Mx^\alpha$ *on* I.

To prove this corollary one sets $R = x^\alpha$ in Corollary 1.

The method suggested in the preceding corollaries is also available for determining bounds for the derivative of a solution $y(x)$ of (1). One simply observes that $z = r(x)y'(x)$ is a solution of the differential equation

$$(p^{-1}z')' + r^{-1}z = 0, \tag{5}$$

which is of the form (1).

Example: To study the differential equation $(3)'$ $y'' + a^2x^{-2}y = 0$ $(4k^2 = 4a^2 - 1 > 0)$ apply Corollary 2 with $\alpha = {}^1/_2$. Thus $|y(x)| \leq Mx^{1/2}$. Equation (5) becomes $(x^2a^{-2}z')' + z = 0$, which under the transformation $z = ax^{-1}w$ becomes $w'' + a^2x^{-2}w = 0$. Thus $|w| \leq Mx^{1/2}$ and $|y'(x)| \leq Mx^{-1/2}$. Solutions of $(3)'$ are readily seen to be $x^{1/2} \sin k \log x$ and $x^{1/2} \cos k \log x$.

The bibliography of this problem is voluminous. Further references to the literature will be found in the list of papers which follows.

Bellman, Richard, "The Boundedness of Solutions of Linear Differential Equations," *Duke Math. J.*, **14**, 83–97 (1947).

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PRINCIPAL QUADRATIC FUNCTIONALS AND SELF-ADJOINT SECOND-ORDER DIFFERENTIAL EQUATIONS

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1. Principal Quadratic Functionals.—A *principal* quadratic functional is defined to be a functional of the form

$$J = \int_0^b [r(x)y'^2 - p(x)y^2]dx \qquad (b > 0), \tag{1}$$

where $r(x)$ and $p(x)$ are continuous and $r(x) > 0$ on the interval $0 < x \leq b$. A function $y(x)$ and the curve $y = y(x)$ are said to be A-admissible[1] on $0 \leq x \leq b$ if

1. $y(x)$ is continuous on $0 \leq x \leq b$ and $y(0) = y(b) = 0$;
2. $y(x)$ is absolutely continuous and $y'^2(x)$ is in L on each closed subinterval of $0 < x \leq b$.

THEOREM 1. *If $p(x) > 0$ when x is near zero and if*

$$\lim_{x \to 0^+} \int_x^b p(x)dx = +\infty,$$

there exists an A-admissible curve $y = y(x)$ along which J assumes a finite negative value.

The above theorem generalizes certain earlier results.[1]

THEOREM 2. *If $p(x) > 0$ near $x = 0$, if there is no point on $0 \leq x < b$ conjugate to $x = 0$, and if*

$$\lim_{x \to 0^+} \int_x^b p(x)dx$$

is finite, then

$$\liminf_{x \to 0^+} \int_x^b (ry'^2 - py^2)dx \geq 0 \tag{2}$$

for all A-admissible functions $y(x)$.

Inasmuch as $x = 0$ is, in general, a singular point of J, the classical definition of the conjugate point is not valid. The definition used here is the generalization of the usual concept which was employed in a paper by Morse and Leighton.[1]

Let $y(x) \not\equiv 0$ be an extremal orthogonal to the line $x = x_0 > 0$. If $x = a$ is the first zero following $x = x_0$, $\lim a$ as $x_0 \to 0+$, when it exists, is called the *focal point of the y-axis*. It can be shown that *if the y-axis contains its focal point, there exists an A-admissible curve $y = y(x)$ along which J assumes a finite negative value.*

The *focal point solution* $y(x)$ of the Euler equation of J may be defined

(when $x = 0$ is not its own first conjugate point). Its smallest positive zero, if it exists, is the focal point of the y-axis, and conversely.

A curve $y = y(x)$ is said to be F-admissible[1] if

1. $y(x)$ is continuous on $(0, b]$ and $y(b) = 0$;
2. $y(x)$ is absolutely continuous and $y'^2(x)$ is in L on each closed subinterval of $(0, b]$.

THEOREM 3. *If $p(x) > 0$ near $x = 0$, the limit (2) is ≥ 0 for all F-admissible curves $y = y(x)$ if and only if there is no focal point of the y-axis on $0 \leq x < b$.*

2. The Oscillation of Solutions of a Self-Adjoint Second-Order Linear Differential Equation; a New Normal Equation.—We study the detection of an infinity of oscillations of the solutions of the equation

$$[r(x)y']' + p(x)y = 0, \tag{3}$$

where $r(x)$, $r'(x)$ and $p(x)$ are continuous and $r(x) > 0$ on $0 < x < \infty$.

The following theorem appears to be fundamental.

THEOREM 4. *If $p(x) > 0$ near $x = +\infty$, and if*

$$\int_1^\infty \frac{dx}{r(x)} = \int_1^\infty p(x)dx = +\infty,$$

every solution of (3) has an infinity of zeros on the interval $1 < x < \infty$. Similarly, if $p(x) > 0$ near $x = 0+$, and if

$$\int_0^1 \frac{dx}{r(x)} = \int_0^1 p(x)dx = +\infty$$

every solution of (3) has an infinity of zeros on the interval $0 < x < 1$.

The proof is elementary.

Roughly speaking the theorem is most sensitive when $\int dx/r(x)$ diverges slowly. This suggests the study of the *normal equation*

$$(xy')' + p(x)y = 0 \tag{4}$$

as superior for many purposes to that of the classical form $y'' + p(x)y = 0$. Equation (3) can be transformed into (4) by the substitution $y = (x/r)^{1/2}z$.

The following result is valid.

THEOREM 5. *If $p(x) > 0$ and $O(x^{-\alpha})(\alpha > 1)$ for x large, a non-null solution of (4) has at most a finite number of zeros on $1 < x < \infty$. Similarly, if $p(x) > 0$ and $O(x^{-\alpha})(\alpha < 1)$ near $x = 0+$, a non-null solution of (4) has at most a finite number of zeros on $0 < x < 1$.*

Detailed proofs of the theorems stated and of others, as well as a more complete bibliography, will be published elsewhere.

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THE MECHANISM OF PROTEINURIA*

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Read before the Academy, November 16, 1948

During the last war the Office of Scientific Research and Development gave this laboratory an opportunity to observe some effects on the kidney of various materials that had been suggested as possible substitutes for blood plasma lost in battle casualties. One of the methods we used was to inject large amounts of the proposed blood substitute into the peritoneal cavities of 150-g. ♀ rats (960 mg. of the material in 6 per cent solution). When a single injection was given we found, in consonance with the work of others,[1] that the rate at which the kidneys excreted any protein depended, first of all, on its molecular weight. Thus proteins whose molecular weight was about 34,000 (egg albumin, globin and Bence Jones protein) were rapidly excreted, proteins with a molecular weight of 70,000 (preparations of bovine or human serum albumin) gave only a slight and delayed increase over the usual amount of protein in the rat's urine, while globulins (molecular weight about 150,000) were not excreted at all. However, when we began to give repeated injections, we found that even proteins with a molecular weight of 70,000 began to appear in the urine until the rate of excretion rose to very high levels and the kidneys were excreting in 24 hours more than their own weight of protein.

Clinicians are called on to treat patients who excrete considerable amounts of protein in their urine. The current supposition is that in these people disease of one sort or another has damaged the glomerular membrane so that it has become permeable to protein. The absence of protein in the urine of healthy people is seen as a consequence of the fact that the normal membrane allows no protein to pass into the urine. This supposition is based on the classical experimental work of Wearn and Richards[2] who first demonstrated that the glomerulus is a filter when they succeeded in obtaining minute amounts of fluid from Bowman's capsule and showed that it was a filtrate of the plasma perfusing the glomerulus, since the concentration of various small molecule solutes was the same in the fluid as in the plasma. This filtrate they found to be "protein-free," or, at least, it contained less than 30 mg. of protein per 100 cc., which was the lowest concentration that could be detected with the most sensitive tests they used. There is thus a sound foundation for the present clinical idea about proteinuria. It might even be regarded as beyond assail if it were not that the volume filtered by a man in 24 hours is about 180,000 cc., so that even if the concentration of protein were only 10 mg. per 100 cc., i.e., too small to be detected, 18,000 mg. would every day leak through the membrane.

We agree that this simple hypothesis as to the mechanism of proteinuria should not be discarded simply because human or bovine proteins of 70,000 molecular weight, do, in the end, and after repeated injections, induce intense proteinuria in the rat. These are "foreign" proteins. We cannot be sure that they may not appear in the urine because they somehow render the glomerular membrane more permeable to protein. It is another matter when we find that proteins from the rat's own blood serum induce the excretion of large quantities of protein, particularly when the injections of serum appear to leave the animals wholly undisturbed. For in this case it would appear that the injections have done no more than increase the concentration of the proteins that are always flowing through the glomerular capillaries. These considerations led us recently to repeat and extend our first observations on the effect of injecting rat serum into rats that we made some years ago during the war.

TABLE 1

PROTEINURIA FOLLOWING EACH INJECTION OF 5 ML. OF RAT SERUM PER 100 SQ. CM OF BODY SURFACE (6.0 G. PROTEIN PER 100 CC.) EVERY 48 HRS. CONTROLS GIVEN THE SAME VOLUME OF 0.85 PER CENT NaCl SOLUTION

		URINE PROTEIN		SERUM PROTEIN	
DAY	INJECTION	RAT SERUM, MG. PER 24 HRS.	NaCl, MG. PER 24 HRS.	RAT SERUM, G. PER 100 CC.	NaCl, G. PER 100 CC.
1st	+	7	2	7.2	5.7
2nd	..	3	3	...	...
3rd	+	19	3	...	...
4th	..	3	2	...	...
5th	+	18	2	8.3	6.1
6th	..	7	2	...	...
7th	+	23	2	...	...
8th	..	5	2	...	...
9th	+	45	2	8.5	5.7

In table 1 we give average 24-hour rates of excretion per rat obtained from two groups, each containing 6 ♀ rats of about 150 g. body weight. The protein was determined by a modified biuret method.[3] One group was given intraperitoneal injections of rat serum and the other, the control group, injections of the same volume of 0.85 per cent NaCl solution.

To us the significance of the data given in table 1 lies in the fact that the slight but very definite and increasing excretion of protein that followed each successive injection of rat serum was found in a group of rats that seemed to be in better health than the control rats given salt solution. At the end of the ten-day period the average control body had fallen from 149 to 138 g. while the rat serum injected group that started at 150 g. had a body weight of 148 g. Both groups were on a protein-free diet of 35 per cent glucose in 0.4 per cent NaCl with 1 per cent of a mixture of B vitamins. Since we have found that the intraperitoneal

injection of horse serum into rats prevents the loss of protein that otherwise occurs on a glucose diet[4] we suppose that the condition of the serum injected rats was better because they were able to use for anabolism some of the rat serum protein we injected. We stress this point because if the injections had disturbed the health of the animals the proteinuria might have been a consequence of damage to the glomerular membrane. It is true, of course, that the injections increased the concentration of protein in the blood. The hematocrit changes indicated that one of the effects was a dilution of the blood in respect of its red blood cell content. But these changes are neither sudden nor extreme, because the absorption of the serum from the peritoneal cavity occurs slowly over a period of several hours. Neither the rate of urine flow nor the blood serum urea concentrations indicated any deviation from normal renal function. This was a pure proteinuria. Under these circumstances we think it would be farfetched to postulate a change in glomerular permeability due to injury. We think that some protein was always escaping through the membrane and that the rate of escape was increased by the increase in serum protein concentration that we induced.

The intensity of the proteinuria induced by rat serum injections is greatly increased if the injections are given at shorter time intervals. When 4 injections of the same volume of serum containing 6.2 g. per 100 cc. are given over a period of 48 hours steeply rising rates of excretion are observed, until after the fourth injection rates corresponding to over 400 mg. of protein per 24 hours are delivered from kidneys that contain only 160 mg. of protein—a rate, in respect of the size of the kidney, higher than we ever obtain from human kidneys no matter to what degree disease may have damaged the glomerular membrane. These very high rates per gram of kidney are associated with unusually high levels of serum protein concentration, sometimes exceeding 12 g. per 100 cc. Here, again, we find no evidence of injury to the kidney, either in the formed elements in the urine or in the histological appearance of the glomeruli. There is, however, an increase in the weight of the kidney, which is due, predominantly, to an increase, not of water, but of protein. Even with lesser degrees of proteinuria this increase in the weight of the kidneys occurs if the injections are reiterated. Thus in the case of the rats whose protein excretion after 5 injections is given in table 1, the salt injected group had kidneys that weighed 833 mg., while the group given rat serum had kidneys that weighed 1042 mg. We believe this weight increase is a consequence of the reabsorption of filtered protein by the proximal tubules.

The rat is not the only animal in which proteinuria can be induced by injection of its own plasma proteins. Thorn and his co-workers found that patients with liver cirrhosis excreted considerable quantities of protein after reiterated intravenous injections of human serum albumin[5] and

recently Whipple and his associates observed in dogs injected with dog plasma long-continued and high urinary excretion rates of albumins and globulins, with no evidence of glomerular damage.[6] Admittedly such observations scarcely warrant positive conclusions. They are too indirect. Their value is negative and lies in the fact that they permit us to doubt the direct and seemingly compelling evidence given by Wearn and Richards that the glomerular filtrate is protein free and the normal glomerular membrane impermeable to serum albumin or any larger plasma protein. Nevertheless that freedom is important because once the idea is entertained that under normal conditions some protein may always be escaping into the urine we find many well-documented clinical, experimental and pathological observations can then for the first time be viewed as consequences of a quantitative intensification of processes that are physiological. We refer to the data on proximal tubule reabsorption of urine protein given by Gérard,[7] Oliver,[8] Dock,[9] Randerath,[10] Smetana,[11] and Yuile and others[12] and to the work of Rather,[13] on the digestion of hemoglobin reabsorbed from the urine. The clinician who has to try to comprehend what he observes in patients with proteinuria finds that there are many questions that have to be laid aside as insoluble so long as he holds to the postulate of the absolute impenetrability to protein of the normal glomerular membrane (capillary wall, basement membrane and epithelial membrane), and continues to believe that all proteinurias arise only because of damage to the membrane. It remains true, of course, that the more complex idea of the mechanism of proteinuria as involving three processes, the filtration, the reabsorption and the intracellular digestion of protein, remains no more than a working hypothesis. But the advantage of the more complex view is that it is susceptible of experimental validation, correction and extension, though that will require the coördinated efforts of groups of workers employing the techniques of physical chemistry, histochemistry and enzyme chemistry, in conjunction with the simpler methods of experimental biology.

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*CRYSTALLOGRAPHIC SYMMETRY IN RECIPROCAL SPACE**

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Communicated by W. J. Mead, February 11, 1949

Reciprocal space constitutes a useful tool for dealing with many diffraction problems,[1] particularly problems concerned with the diffraction of x-rays by crystals. Wrinch[2] has shown that if a distribution has a point-group symmetry element through the origin, its Fourier transform in reciprocal space has the same symmetry element. For crystallographic purposes, it is desirable to go farther than this and inquire how the more general crystallographic symmetry elements appear in reciprocal space.

Consider a symmetry element with a translation component, such as A, an n-fold screw through the origin, O, figure 1. Let the screw, for definiteness, lie along the crystallographic c axis. The figure shows two sets of planes related by an operation of the screw. One set of planes may be indexed hkl, the other $h'k'l'$, but both have the common interplanar spacing d_{hkl}. In reciprocal space, these sets of planes are represented by points P and P', respectively. P lies on the normal to hkl, and P' lies on the normal to $h'k'l'$, both normals extending from the origin. Both P and P' have the same origin distance, namely $1/d_{hkl}$. Since the direction of the normal is unaffected by the displacement of the planes due to the translational component of the axis, it is evident that the rotational component of the crystal symmetry element is preserved in the relative locations of the points hkl and $h'k'l'$ in reciprocal space.

The relative phases of the two points, however, depend on the relative displacements of the two sets of planes from the origin.[1] In figure 1, the phase difference in reciprocal space corresponding with the displacement between two neighboring planes of the set hkl is 2π. This difference can be measured by the displacement of the planes along the c axis. Since there are l planes per unit translation along the c axis, the total phase

difference per unit translation along the c axis is $2\pi l$. However, the translation component of the screw produces a displacement of planes only a fraction of a translation, namely $(1/q)c$, so that the phase difference due to this translation component is $2\pi l/q$.

Let the reciprocal at P be F_{hkl}. In general, the phase of F_{hkl} is not zero, but some value, say φ. Thus the phase component of F_{hkl} in the complex plane is $e^{2\pi i\varphi}$. Then the phase component at P' according to the last paragraph is $e^{2\pi i\varphi}e^{2\pi il/q}$. Since the absolute magnitudes at P and P' are identical, the reciprocals at these two points must be related as follows:

$$F_{h'k'l'} = F_{hkl}e^{2\pi il/q},$$

where c/q is the translation component of the symmetry operation. Note that the phase difference vanishes for symmetry elements without a translation component (i.e., $q = 0$), and also for indices such that $l/q =$ an integer.

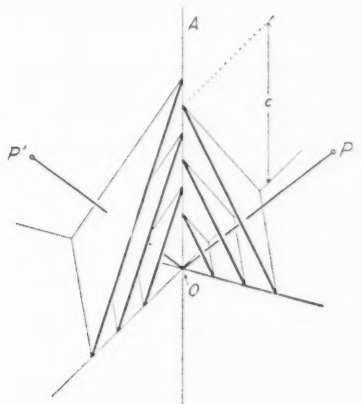

FIGURE 1

By permitting the rotational component of the symmetry element to include improper rotations, this result can be extended to include symmetry operations of the second sort. Alternatively, corresponding results can be derived directly for reflections, glide-reflections and roto-inversions. Neither the sequence of planes from the origin nor their distances away from the origin are affected by the improper character of the rotation, so that the only effect that the nature of the rotation has on reciprocal space is with respect to the location of the point.

As a consequence of these considerations, the following statement can be made:

THEOREM 1. *If a crystal contains a symmetry element through a chosen*

origin in direct space, its reciprocal has the isomorphous (translation-free) symmetry element at the origin, but the points in the fields related by the operations of the symmetry element have phases related by $e^{2\pi ip/q}$, *where* p *is the power of the operation relating the fields and* a/q *is the translation component of the operation.*

(For diagonal symmetry elements having the general translation component $(a + b + c)/q$, it can be shown that $(h + k + l)$ should be substituted for h.)

In case the symmetry axis does not contain the origin, but is displaced to a location having coördinates xa, yb, the planes hkl (along with their symmetrically equivalent planes) are displaced too, as shown in figure 2. Since there are h planes along a, and each separation corresponds with a phase difference in reciprocal space of 2π, a displacement of the entire a translation corresponds to $2\pi h$. Therefore, the component of the displacement of the axis along a, of amount xa, corresponds with a phase difference of $2\pi xh$. Similarly, the component of its displacement, of

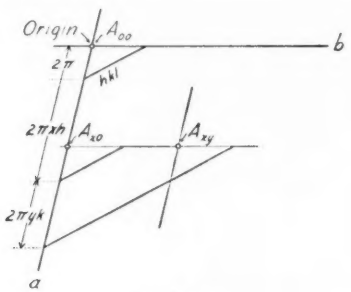

FIGURE 2

amount yb, along b corresponds with a phase difference of $2\pi yk$. Consequently, the entire displacement of the axis from the origin to location xa, yb corresponds with a phase difference of $2\pi(xh + yk)$ applied to F_{hkl} in reciprocal space. Symbolically,

$$_{x,y}F_{hkl} = {}_{0,0}F_{hkl}e^{2\pi i(xh + yk)}.$$

THEOREM 2. *If a symmetry element is displaced by an amount* $xa + yb$, *the phase component difference produced on* F_{hkl} *accompanying this displacement is* $e^{2\pi i(xh + yk)}$.

Space groups can be developed by adding to the lattice operations various generating operations isomorphous with the point-group operations. In the reciprocal of the space group, the translations of the lattice are responsible for limiting the non-vanishing values of the Fourier transform

of the crystal structure to the points of the reciprocal lattice in reciprocal space. The generating symmetry operations and their locations are responsible for fixing the relative phases at the points of the reciprocal lattice which are equivalent by the point-group symmetry.

THEOREM 3. *For each generating operation of the cell of the space group, there is a symmetry relation between points in reciprocal space: The position and absolute-magnitude symmetry is the same as that of the point group isomorphous with the space group, non-vanishing values occurring only at points of the lattice reciprocal to the lattice of the crystal, while the phases of the points related by this symmetry are governed by the translation components and positions of the symmetry elements as well as the indices of the points (in accordance with Theorems 1 and 2).*

These theorems make it possible to write down by inspection, all the relations between F's for a given space group for any chosen origin. The customary method of obtaining such information is the laborious structure-factor method.[3] Theorem 2 also provides a tool for predicting the changes in phase relations which accompany any displacement of the origin of the space group.

A space group is determined by the lattice type, the point group and the generating operations isomorphous with the point-group operations. Since each of these has a unique representation in reciprocal space for a chosen origin, it is evident that all space groups can be distinguished by their representations in reciprocal space.

* This paper contains the essence of a lecture given at the Faculdade Nacional de Filosofia, Rio de Janeiro, December 24, 1948, on the subject, "Reciprocal Space."

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*PHYSICAL CURVES IN SPACE OF n DIMENSIONS**

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1. A *system* S_k of ∞^{2n-1} curves in a given positional field of force[1] in Euclidean space of n dimensions consists of curves along which a constrained motion is possible such that the osculating plane at each point contains the force vector F, and the pressure P (along the principal normal

to the curve) is proportional to the normal component N of F. Thus $P = kN$ where $k(\neq -1)$ is the constant of proportionality.[2]

The vector differential equation of a system S_k of ∞^{2n-1} curves is

$$\ddot{x} = (1 + k)F - \frac{k(F\dot{x})}{\dot{x}^2}\dot{x}. \tag{1}$$

2. Let $\lambda_1, \lambda_2, \ldots, \lambda_n$, be the mutually orthogonal set of principal unit vectors, such that the scalar product $(\lambda_1, \lambda_2, \ldots, \lambda_n)$ is unity, at a point $x = x^\alpha (\alpha = 1, \ldots, n)$ of a curve C. The Serret-Frenet formulas are

$$\frac{d\lambda_\beta}{ds} = -\kappa_{\beta-1}\lambda_{\beta-1} + \kappa_\beta\lambda_{\beta+1}, \tag{2}$$

for $\beta = 1, \ldots, n$. Of course, $\kappa_0 = 0$ and $\kappa_n = 0$. The quantities $\kappa_1, \kappa_2, \ldots, \kappa_{n-1}$ are the $(n - 1)$ curvatures of C at x.

By means of (2), it is found that *a system of S_k of ∞^{2n-1} curves, as defined by (1), is given by the equations*

$$\dot{v} = T, \qquad v^2\kappa_1 = (1 + k)N, \qquad F = T\lambda_1 + N\lambda_2, \tag{3}$$

where $T = (F\lambda_1)$ and $N = (F\lambda_2)$.

Eliminating the speed v from (3), we find that *the intrinsic equations of a system S_k of ∞^{2n-1} curves are*

$$N\frac{d\kappa_1}{ds} = \left(\frac{dF}{ds}\lambda_2\right)\kappa_1 - \frac{3 + k}{1 + k}T\kappa_1^2, \qquad F = T\lambda_1 + N\lambda_2. \tag{4}$$

The rate of variation $d\rho_1/ds$ of the first radius of curvature $\rho_1 = 1/\kappa_1$ per unit length of arc is a linear integral function of ρ_1 with coefficients depending on x, λ_1, λ_2 only. That is,

$$N\frac{d\rho_1}{ds} = -\left(\frac{dF}{ds}\lambda_2\right)\rho_1 + \frac{3 + k}{1 + k}T. \tag{5}$$

3. Consider any curve C along which a constrained motion is possible such that the osculating plane at each point of C contains the force vector F. We denote the totality of all such curves C by Σ. Thus S_k is a subset of Σ.

At each point of a curve C of Σ, the osculating m flat where $m < n$ contains the vector $d^{m-2}F/ds^{m-2}$.

All the curves C of Σ which pass through a given point in a given direction have the same osculating plane and the same osculating three flat at this point provided that $N \neq 0$. The osculating m flat where $m = 4, \ldots, n - 1$, varies.

Let $N \neq 0$. The second curvature κ_2 is a function of x, λ_1, λ_2 only. If

$\kappa_2 \neq 0$, the third curvature κ_3 is the square root of a quadratic polynomial in κ_1 with coefficients functions of x, λ_1, λ_2 only.

4. Consider the curvatures $\kappa_1, \kappa_2, \ldots, \kappa_{n-1}$ of the curves of a system S_k. As S_k is a special case of Σ, it is seen that κ_2 and κ_3 have the properties described above.

In general, *the m-th curvature κ_m for $m = 3, 4, \ldots, n-1$, of any curve C of a system S_k, is the square root of a polynomial in κ_1 divided by a polynomial in κ_1.*

The initial curvatures of the ∞^1 curves of a system S_k which pass through a fixed point in a fixed direction obey the following laws. The first curvature κ_1 varies inversely as the square of the speed v. The second curvature κ_2 is constant. The third curvature κ_3 is the square root of a quadratic polynomial of κ_1. The fourth curvature κ_4 is the square root of a fourth degree polynomial of κ_1 divided by the quadratic polynomial appearing in κ_3. The fifth curvature κ_5 is the square root of a tenth degree polynomial of κ_1 divided by the fourth degree polynomial appearing in κ_4.

5. If a particle starts from rest, the resulting curve of the system S_k is called a *rest trajectory*. The vector $d^{m-1}F/ds^{m-1}$ is in the osculating m flat for $m = 1, \ldots, n-1$, at the initial point of a rest trajectory.

The rest trajectory and the line of force are initially tangent and possess the same osculating m flat for $m = 2, 3, \ldots, n-1$.

The vector dF/ds for the rest trajectory coincides with the vector dF/ds for the line of force. However, for $m = 3, 4, \ldots$, the vector $d^{m-1}F/ds^{m-1}$ constructed for the rest trajectory is distinct from the corresponding vector $d^{m-1}F/ds^{m-1}$ constructed for the line of force. These two vectors are situated in the same m flat.

The ratio of the first curvature of the rest trajectory to the first curvature of the line of force is

$$\frac{1+k}{3+k}. \tag{6}$$

For dynamical trajectories ($k = 0$), this ratio is $1/3$, a well-known result. For general catenaries ($k = 1$), this ratio is $1/2$. For brachistochrones ($k = -2$), this ratio is -1. For velocity curves ($k = \infty$), this ratio is $+1$.

There is no analogous such formula for the second curvatures or torsions, or for the remaining curvatures.[3]

6. The center S of the osculating m sphere of a curve C is

$$S = x + \rho_1\lambda_2 + \rho_2\frac{d\rho_1}{ds}\lambda_3 + \rho_3\left[\frac{\rho_1}{\rho_2} + \frac{d}{ds}\left(\rho_2\frac{d\rho_1}{ds}\right)\right]\lambda_4 + \ldots + \sigma_{m+1}\lambda_{m+1}, \tag{7}$$

where $\rho_1, \ldots, \rho_{n-1}$ are the $(n-1)$ radii of curvature and the various coefficients may be obtained by the recursion formula

$$\sigma_p = \rho_{p-1}\left[\frac{\sigma_{p-2}}{\rho_{p-2}} + \frac{d\sigma_{p-1}}{ds}\right] \tag{8}$$

for $p = 2, 3, \ldots, m + 1$.

The locus of the centers of the osculating m spheres of the ∞^1 *curves of a system* S_r *which pass through a fixed point in a fixed direction is an algebraic curve whose position vector* S *is an algebraic function of the first curvature* $\kappa_1 = 1/\rho_1$.

For $m = 2$, *this locus is a straight line, just as is the case for* $n = 3$. This line is given by the system of $(n - 1)$ equations

$$\lambda_1(S - x) = 0, \qquad \lambda_4(S - x) = 0, \qquad \ldots, \qquad \lambda_n(S - x) = 0,$$

$$(1 + k)\frac{dF}{ds}(S - x) = (3 + k)(F\lambda_1). \tag{9}$$

To each direction λ_1 through the fixed point x, there corresponds a straight line (9). The line which corresponds to the direction of the force vector acting at the point x is parallel to the binormal of the line of force. Its distance from the point x is $(3 + k)/(1 + k)$ times the radius of curvature of the line of force.

This distance from the fixed point x is equal to the radius of curvature of the rest trajectory through the fixed point. Hence the above line passes through the center of circular curvature of the rest trajectory, a new general theorem for all physical families.

* Presented to the American Mathematical Society, 1949.

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*ON BENDING OF CURVED THIN-WALLED TUBES**

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1. Introduction.—The present note is concerned with the problem of pure bending of curved tubes. The fact that a curved thin-walled tube is more flexible than a straight tube of the same cross-section has first been explained by von Kármán[1] who showed that the flattening of the

tube cross-sections due to the action of the primary bending stresses is responsible for this effect. Von Kármán's approximate solution of the problem makes use of the minimum principle for the displacements in elasticity in Rayleigh-Ritz fashion. Subsequently H. Lorenz[2] gave an alternate approximate solution by the use of the minimum principle for the *stresses* in elasticity. This same method was also used by L. Beskin[3] in a recent paper on the subject.

In what follows we propose to consider the problem from the point of view of the theory of thin elastic shells. Inasmuch as the bending of a curved thin tube may be thought of as a problem of determining an axisymmetrical stress distribution in a shell of revolution it must be possible to relate the resultant formulation of the problem to the results of H. Reissner[4] and E. Meissner[5] on the bending and stretching of shells of revolution. H. Reissner, E. Meissner and their followers restricted attention to problems in which not only stresses but also displacements are rotationally symmetric and univalued. Evidently the latter assumption excludes the problem of tube bending from their results. In what follows we make the necessary slight generalization of the theory such that a system of equations is obtained which contains new terms originating from a suitably chosen multivalued expression for one of the components of displacement. We may remark that in so doing we prepare the way for treating the problem of pure tube bending in a manner which is analogous to M. Golovin's treatment[6] of pure bending of a circular ring sector of uniform thickness according to the theory of plane stress.

2. Basic Relations for Axisymmetrical Deformations of Thin Shells of Revolutions.—Let $r = r(\xi)$, $z = z(\xi)$ be the equations of the middle surface of the shell in parametric form and write

$$\alpha^2 = (r')^2 + (z')^2, \qquad r' = \alpha \cos \phi, \qquad z' = \alpha \sin \phi, \tag{1}$$

where primes indicate differentiation with respect to ξ and where ϕ is the tangent angle to a meridian curve. Let u and w be components of displacement in *radial* and *axial* direction, respectively, and let β be the change of sloping angle due to deformation. It can then be shown that for small deformations and under the assumption that normals to the middle surface are preserved during deformation the state of strain in the deformed shell may be described as follows:

$$\epsilon_\xi = \epsilon_{\xi M} + \zeta \kappa_\xi, \qquad \epsilon_\theta = \epsilon_{\theta M} + \zeta \kappa_\theta, \tag{2}$$

where ζ is the distance from the middle surface and where

$$\left.\begin{aligned} \epsilon_{\xi M} &= \frac{u'}{r'} - \beta \tan \phi, \qquad \kappa_\xi = \frac{\beta'}{\alpha} \\ \epsilon_{\theta M} &= \frac{u}{r}, \qquad \kappa_\theta = \frac{r'}{r}\frac{\beta}{\alpha}. \end{aligned}\right\} \tag{3}$$

Equations (3) imply a compatibility relation of the form

$$(r\epsilon_{\theta M})' - r'\epsilon_{\xi M} = z'\beta. \tag{4}$$

Let H, V, N_ξ, N_θ and Q be stress results, let M_ξ and M_θ be stress couples and let p_H and p_V be components of load intensity. We then have the following equilibrium equations:

$$\left.\begin{array}{c} (rV)' + r\alpha p_V = 0 \\ (rH)' - \alpha N_\theta + r\alpha p_H = 0 \\ (rM_\xi)' - r'M_\theta + z'(rH) - r'(rV) = 0 \\ r\alpha N_\xi = r'(rH) + z'(rV) \\ r\alpha Q = -z'(rH) + r'(rV). \end{array}\right\} \tag{5}$$

Equations (5) together with appropriate stress-strain relations are a form of the basic equations from which the results of H. Reissner and E. Meissner may be deduced.[7] Before indicating the way in which this is done we shall introduce into (3) to (5) the modification which is necessary to include the problem of pure tube bending.

3. Introduction of Multivalued Circumferential Displacement Component.—Equations (3) for the relevant components of strain were obtained under the assumption of vanishing circumferential displacement component v. We now assume instead that

$$v = kr\theta, \tag{6}$$

where k is a constant and r and θ are polar coördinates in the x, y-plane. Note that when v is given by (6) and $u = w = 0$ then the components of strain in cylindrical coördinates have the values $\epsilon_r = \epsilon_z = \gamma_{r\theta} = \gamma_{rz} = \gamma_{\theta z} = 0$ and $\epsilon_\theta = k$. This means that the effect of replacing the condition $v = 0$ by (6) leaves $\epsilon_{\xi M}$, κ_ξ and κ_θ of equations (3) unchanged and makes

$$\epsilon_{\theta M} = \frac{u}{r} + k. \tag{3'}$$

In view of (3') the compatibility equation (4) is changed to

$$(r\epsilon_{\theta M})' - r'\epsilon_{\xi M} = z'\beta + r'k. \tag{4'}$$

4. The Differential Equations of the Bending Problem.—The procedure from here on is the same as that for the case $k = 0$, that is, two simultaneous second-order equations are derived for the quantities rH and β. In doing this we shall assume an orthotropic tube material with the following stress-strain relations:

$$\epsilon_{\xi M} = \frac{N_\xi}{C_\xi} - \frac{N_\theta}{C_{\xi\theta}}, \qquad \epsilon_{\theta M} = \frac{N_\theta}{C_\theta} - \frac{N_\xi}{C_{\xi\theta}} \tag{7}$$

$$M_\xi = D_\xi\kappa_\xi + D_{\xi\theta}\kappa_\theta, \qquad M_\theta = D_\theta\kappa_\theta + D_{\xi\theta}\kappa_\xi. \tag{8}$$

For the present problem we may in equations (5) set $p_N = p_H = V = 0$. We combine (7) and (4′) and use (5) to obtain the first of the two simultaneous equations in the following form:

$$(rH)'' + \frac{(r/\alpha C_\theta)'}{(r/\alpha C_\theta)}(rH)' - \left[\frac{C_\theta}{C_\xi}\left(\frac{r'}{r}\right)^2 + \frac{(r'/\alpha C_{\xi\theta})'}{(r/\alpha C_\theta)}\right](rH) = \frac{z'\beta + r'k}{(r/\alpha C_\theta)}. \tag{I}$$

Next we combine (8) and (3) and introduce the result into the third of equations (5) to obtain the second of the two simultaneous equations in the following form:

$$\beta'' + \frac{(rD_\xi/\alpha)'}{(rD_\xi/\alpha)}\beta' - \left[\frac{D_\theta}{D_\xi}\left(\frac{r'}{r}\right)^2 - \frac{(r'D_{\xi\theta}/\alpha)'}{(rD_\xi/\alpha)}\right]\beta = -\frac{z'(rH)}{(rD_\xi/\alpha)}. \tag{II}$$

Equations (I) and (II) must be integrated, subject to suitable boundary conditions for open tubes or periodicity conditions for closed tubes. A relation between k and the applied bending moment m is obtained, after (I) and (II) are solved, by means of the equation defining m,

$$m = \int rN_\theta \alpha d\xi - \int M_\theta z' d\xi. \tag{9}$$

5. Some Special Cases.—Equations corresponding to von Kármán's assumptions are obtained by setting

$$C_\xi = C_{\xi\theta} = \infty, \qquad D_\theta = D_{\xi\theta} = 0. \tag{10}$$

With these assumptions equation (I) may be reduced to the form

$$[(r/\alpha C_\theta)(rH)']' = z'\beta + r'k, \tag{11}$$

while equation (II) may be reduced to

$$[(rD_\xi/\alpha)\beta']' = -z'(rH). \tag{12}$$

An equation governing the bending of a circular ring sector is obtained from (I) by setting

$$r = \xi, \qquad z = 0, \qquad \alpha = 1, \tag{13}$$

and is of the form

$$(rH)'' + \frac{(r/C_\theta)'}{(r/C_\theta)}(rH)' - \left[\frac{C_\theta}{C_\xi}\frac{1}{r^2} + \frac{(1/C_{\xi\theta})'}{(r/C_\theta)}\right](rH) = \frac{C_\theta}{r}k. \tag{14}$$

When $C_\theta = C_\xi = Eh = \text{const.}$ and $C_{\xi\theta} = Eh/\nu = \text{const.}$ equation (14) leads to Golovin's solution[6] of the problem. When $C_\theta = Eh = \text{const.}$ and $C_{\xi\theta} = C_\xi = \infty$ then equation (14) leads to the hyperbolic distribution of N_θ first obtained by Winkler and Resal.[6]

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*NOTE ON THE THEORY OF LIFTING SURFACES**

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1. Introduction.—In this note we consider the problem of the steady motion of a rectangular lifting surface of finite span in incompressible flow. We are concerned with two separate objects. One is an attempt to derive somewhat more clearly than in the original paper Weissinger's improvement of Prandtl's lifting-line theory.[1] The other is to generalize the one-dimensional integral equation of lifting-line theory for the spanwise variation of lift intensity to two simultaneous equations for lift intensity and moment intensity. In so doing, certain difficulties of lifting-line theory and of its improvements by Weissinger with reference to behavior at and near the tips of the wing are substantially ameliorated.

While we restrict attention here to a rectangular surface and to incompressible flow, the extension to subsonic compressible flow is a simple matter, and also the extension to tapered wings with or without sweep is feasible.

2. The integral equation of the rectangular lifting surface.—We consider a nearly plane wing with chord $2b$ and span $2sb$. The integral equation of the linearized theory may be written in the following dimensionless form:[2]

$$w(x, y) = \frac{1}{\pi}\int_{-1}^{1}\frac{p(\xi, y)}{x-\xi}\,d\xi + \frac{1}{2\pi}\int_{-s}^{s}\int_{-1}^{1}\frac{\partial p}{\partial \eta}\left\{\frac{1}{y-\eta} + K(x-\xi, y-\eta)\right\}d\xi d\eta. \quad (1)$$

In equation (1) w is proportional to the slope $\partial h/\partial x$ of the lifting surface, and p is the pressure jump across the lifting surface. Of all singular integrals the Cauchy principal value is to be taken. The function K

is of the form

$$K = \frac{\sqrt{(x-\xi)^2 + (y-\eta)^2} - |y-\eta|}{(x-\xi)(y-\eta)}. \tag{2}$$

The problem of lifting surface theory consists in the solution of (1) for $p(x, y)$ in the region $(-1 \leqslant x \leqslant 1; \quad -s \leqslant y \leqslant s)$ subject to the trailing edge condition that $p(1, y)$ remains finite.

3. Lifting-Line Theory.—Lifting-line theory may be thought of as an approximate solution of (1), in the following sense. The term Ks in the integral equation is neglected, essentially on the basis of the fact that for sufficiently large values of the aspect ratio sK behaves like $1/(y-\eta)\,|\,y-\eta\,|$ and is therefore small compared with $1/(y-\eta)$ over most of the range of integration.

To obtain the lifting-line equation one may multiply the abbreviated equation (1) by a factor $[(1+x)/(1-x)]^{1/2}$ and integrate as follows:

$$\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, w dx = \frac{1}{\pi}\int_{-1}^{1} p(\xi, y) \left\{\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, \frac{dx}{x-\xi}\right\} d\xi$$

$$+ \frac{1}{2\pi}\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, dx \int_{-s}^{s} \frac{d}{d\eta}\left(\int_{-1}^{1} p d\xi\right)\frac{d\eta}{y-\eta}. \tag{3}$$

Let

$$l(y) = \int_{-1}^{1} p(\xi, y) d\xi \tag{4}$$

and use the formula

$$\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, \frac{dx}{x-\xi} = \pi. \tag{5}$$

Equation (3) then assumes the following form:

$$\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, w dx = l(y) + \frac{1}{2}\int_{-s}^{s} \frac{dl}{d\eta}\, \frac{d\eta}{y-\eta}. \tag{6}$$

One disadvantage of this theory consists, for wings with finite tip chord, in the fact that, while the lift intensity assumes the correct values zero at the tips, the pressures $p(x, \pm s)$ do not, in general, and the results of the theory will be of doubtful validity in regions adjacent to the tips, presumably up to distances inward from the tips of the order of magnitude of the tip chord.

This behavior of the lifting-line theory may be seen by a consideration of the section moment m, defined as

$$m(y) = \int_{-1}^{1} p(\xi, y)\xi d\xi. \tag{7}$$

It can be shown as follows that, in general, $m(\pm s)$ does not assume the value zero which it would have in the solution of the complete equation

(1). We multiply the abbreviated equation (1) by a factor $(1 - x^2)^{1/2}$ and integrate again across the chord,

$$\int_{-1}^{1} \sqrt{1-x^2}\, w dx = \frac{1}{\pi}\int_{-1}^{1} p(\xi, y)\left\{\int_{-1}^{1} \frac{\sqrt{1-x^2}}{x-\xi}\, dx\right\} d\xi + \frac{1}{2\pi}\int_{-1}^{1} \sqrt{1-x^2}\, dx \int_{-s}^{s} \frac{dl}{d\eta}\frac{d\eta}{y-\eta}. \quad (8)$$

With (7) and with the relation

$$\int_{-1}^{s1} \frac{\sqrt{1-x^2}}{x-\xi}\, dx = -\pi\xi,$$

we may write (8) in the form

$$\int_{-1}^{1} \sqrt{1-x^2}\, w dx = -m(y) + \frac{1}{4}\int_{-s}^{s} \frac{dl}{d\eta}\frac{d\eta}{y-\eta}. \quad (10)$$

Combination of (10) and (6) gives the following alternate expression for m,

$$m = -\frac{1}{2}l + \frac{1}{2}\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, w dx - \int_{-1}^{1} \sqrt{1-x^2}\, w dx. \quad (11)$$

In general the second and third terms in (11) do not cancel each other and consequently we have in general $m(\pm s) \neq 0$. A known exception forms the case of constant $w(x, \pm s)$ that is the case of a wing with uncambered tip chord.

4. Weissinger's Extension of the Lifting-Line Theory.—This extension takes into account in an approximate manner the term with K in equation (1). It may be derived in the following way. Corresponding to (6) and in the same manner, the following exact relation is first obtained

$$\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, w dx = l(y) + \frac{1}{2}\int_{-s}^{s} \frac{dl}{d\eta}\frac{d\eta}{y-\eta} + \frac{1}{2}\int_{-s}^{s}\int_{-1}^{1} \frac{\partial p}{\partial \eta}\left\{\frac{1}{\pi}\int_{-1}^{1} \sqrt{\frac{1+x}{1-x}}\, K(x-\xi, y-\eta) dx\right\} d\xi d\eta. \quad (12)$$

The last term on the right of (12) is to be taken into account approximately only and this is done by stipulating that in it one may set

$$p(\xi, \eta) = \frac{l(\eta)}{\pi}\sqrt{\frac{1-\xi}{1+\xi}}, \quad (13)$$

which is the exact solution when w is constant and the flow is two-dimensional. Taking as an abbreviation for the triple integral in (12) the symbol I we have then

$$I = \frac{1}{2}\int_{-s}^{s} \frac{dl}{d\eta}\left\{\frac{1}{\pi^2}\int_{-1}^{1}\int_{-1}^{1}\sqrt{\frac{1-\xi}{1+\xi}}\sqrt{\frac{1+x}{1-x}}\,K(x-\xi, y-\eta)d\xi dx\right\}d\eta$$

$$= \frac{1}{2}\int_{-s}^{s}\frac{dl}{d\eta}\,\tilde{K}(y-\eta)d\eta. \quad (14)$$

Equations (12) and (14) are not yet Weissinger's results but rather, in principle, an improvement of his results for the rectangular plan-form wing.

Weissinger's first result, called the F-method, is obtained by introducing the following approximation

$$\frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}\,K(x-\xi, y-\eta)dx \approx K\left(\frac{1}{2}-\xi, y-\eta\right). \quad (15)$$

Note that equation (15) would be exact if K were a linear function of x. With (15) we have further

$$\tilde{K}(y-\eta) \approx \frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1-\xi}{1+\xi}}\,K\left(\frac{1}{2}-\xi, y-\eta\right)d\xi = K_F. \quad (16)$$

The function K_F can be expressed in terms of elliptic integrals and has been tabulated by Weissinger.

Evidently, once the approximation (15) has been made it is only consistent to make a corresponding approximation in (16). This leads to the so-called L-method which in Weissinger's paper is based on lifting-line considerations. The result is

$$\tilde{K}(y-\eta) \approx K(1, y-\eta) = K_L(y-\eta). \quad (17)$$

Calculations have shown[1] that results based on either K_F or K_L are practically indistinguishable. One advantage of K_L besides its simplicity is the fact that an analogous result may be obtained without too much difficulty for wings with sweepback.[1]

With the approximation (17) the integral equation (12) may be written in the following form

$$\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}\,w dx$$

$$= l(y) + \frac{1}{2}\int_{-s}^{s}\frac{dl}{d\eta}\,\frac{1+\sqrt{1+(y-\eta)^2}-|y-\eta|}{y-\eta}\,d\eta. \quad (18)$$

Equation (18) shows somewhat more clearly than the corresponding result in Weissinger's paper the relation between this theory and the original lifting-line theory. It also shows, since $[1+(y-\eta)^2]^{1/2} >$

$|y - \eta|$, that the improved theory leads to more pronounced aspect ratio effects than the original theory.

We may note that with

$$l(y) = \sqrt{1 - y^2} \sum l_n y^n \tag{19}$$

the integrals on the right are expressible in terms of elliptic integrals. It is questionable, however, whether to do so will be of computational advantage.

We remark finally that here also, as in the lifting-line equations (6) and (11), it can be shown that in general the tip moments $m(\pm s)$ do not vanish.

5. A System of Simultaneous Equations for Lift and Moment Distribution.—We begin with the exact equation (12) and add to this the following exact equation which takes the place of the approximate equation (10)

$$\int_{-1}^{1} \sqrt{1 - x^2}\, w dx = -m(y) + \frac{1}{4} \int_{-s}^{s} \frac{dl}{d\eta} \frac{d\eta}{y - \eta} + \frac{1}{2} \int_{-s}^{s} \int_{-1}^{1} \frac{\partial p}{\partial \eta} \left\{ \frac{1}{\pi} \int_{-1}^{1} \sqrt{1 - x^2}\, K dx \right\} d\xi d\eta. \tag{20}$$

We must now take into account approximately the triple integrals both in (12) and (20). As we wish to express these triple integrals both in terms of l and m we can now no longer use the simple approximation (13) for p. Instead we set

$$p(\xi, \eta) = [c_1(1 - \xi) + c_2(1 - \xi^2)]/\sqrt{1 - \xi^2} = -\frac{2m(\eta)}{\pi} \sqrt{\frac{1 - \xi}{1 + \xi}} + \frac{2l(\eta) + 4m(\eta)}{\pi} \sqrt{1 - \xi^2}. \tag{21}$$

With (21) we obtain from (12) and (20) the following two fundamental equations

$$\int_{-1}^{1} \sqrt{\frac{1 + x}{1 - x}}\, w dx = l(y) + \int_{-s}^{s} \frac{dl}{d\eta} \left[\frac{1}{2(y - \eta)} + K_{ll}(y - \eta) \right] d\eta + \int_{-s}^{s} \frac{dm}{d\eta} K_{lm}(y - \eta) d\eta \tag{22}$$

$$\int_{-1}^{1} \sqrt{1 - x^2}\, w dx = -m(y) + \int_{-s}^{s} \frac{dl}{d\eta} \left[\frac{1}{4(y - \eta)} + K_{ml}(y - \eta) \right] d\eta + \int_{-s}^{s} \frac{dm}{d\eta} K_{mm}(y - \eta) d\eta. \tag{23}$$

The kernel functions K_{ll}, etc., are seen to be of the form

$$K_{ll} = \frac{1}{\pi^2} \int_{-1}^{1} \int_{-1}^{1} \sqrt{\frac{1 + x}{1 - x}} \sqrt{1 - \xi^2} K dx d\xi \tag{24}$$

$$K_{lm} = \frac{1}{\pi^2}\int_{-1}^{1}\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}\left[2\sqrt{1-\xi^2} - \sqrt{\frac{1-\xi}{1+\xi}}\right]K dx d\xi \quad (25)$$

$$K_{ml} = \frac{1}{\pi^2}\int_{-1}^{1}\int_{-1}^{1}\sqrt{1-x^2}\sqrt{1-\xi^2}\,K dx d\xi = 0 \quad (26)$$

$$K_{mm} = \frac{1}{\pi^2}\int_{-1}^{1}\int_{-1}^{1}\sqrt{1-x^2}\left[2\sqrt{1-\xi^2} - \sqrt{\frac{1-\xi}{1+\xi}}\right]K dx d\xi = -K_{ll}. \quad (27)$$

The two functions K_{ll} and K_{lm} may be tabulated, just as the function K_F of equation (16) has been tabulated and just as the function $\tilde{K}$ in (14) may be tabulated. Once this has been done one may solve equations (22) and (23) by means of series of the form

$$l = \sum l_k \sin k\phi, \qquad m = \sum m_k \sin k\phi \quad (28)$$

where

$$\cos\phi = y/s. \quad (29)$$

Equations (22) and (23) then become systems of simultaneous equations for the coefficients l_k and m_k. For specific applications it will be necessary to tabulate the functions

$$G_{ll}^{k}(s, \cos\phi) = \int_0^{\pi} \cos k\theta K_{ll}[s(\cos\phi - \cos\theta)]d\theta \quad (33)$$

and corresponding expressions for K_{lm}, for various values of k and s, whence the usual collocation procedure can be applied; or to tabulate the corresponding quantities which occur in an application of the Multhop procedure to equations (22) and (23).

Considerable simplification is introduced into the foregoing results if again as in going from (15) to (16) and (17) use is made of the following formulae for approximate numerical integration

$$\left.\begin{aligned} \frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}f(x)dx \approx f\left(\frac{1}{2}\right), \qquad \frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1-x}{1+x}}f(x)dx \approx f\left(-\frac{1}{2}\right) \\ \frac{1}{\pi}\int_{-1}^{1}\sqrt{1-x^2}f(x)dx \approx \frac{1}{2}f(0). \end{aligned}\right\} \quad (34)$$

Equations (24) and (25) then become

$$K_{ll} \approx \frac{1}{2}K\left(\frac{1}{2}, y-\eta\right) = \frac{\sqrt{1/4 + (y-\eta)^2} - |y-\eta|}{y-\eta} \quad (35)$$

$$K_{lm} \approx K\left(\frac{1}{2}, y-\eta\right) - K\,(1, y-\eta)$$
$$= \frac{\sqrt{1+4(y-\eta)^2} - \sqrt{1+(y-\eta)^2} - |y-\eta|}{y-\eta}. \quad (36)$$

More accurate approximations to K_{ll} and K_{lm} may be obtained if instead of equations (34) the following approximate numerical integration formulae are used

$$\left.\begin{aligned}
\frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}f(x)dx &\approx \frac{5+\sqrt{5}}{10}f\left(\frac{1+\sqrt{5}}{4}\right) + \frac{5-\sqrt{5}}{10}f\left(\frac{1-\sqrt{5}}{4}\right)\\
\frac{1}{\pi}\int_{-1}^{1}\sqrt{\frac{1-x}{1+x}}f(x)dx &\approx \frac{5+\sqrt{5}}{10}f\left(-\frac{1+\sqrt{5}}{4}\right)\\
&\quad + \frac{5-\sqrt{5}}{10}f\left(-\frac{1-\sqrt{5}}{4}\right)\\
\frac{2}{\pi}\int_{-1}^{1}\sqrt{1-x^2}f(x)dx &\approx \frac{1}{2}f\left(\frac{1}{2}\right) + \frac{1}{2}f\left(-\frac{1}{2}\right).
\end{aligned}\right\} \quad (37)$$

While equations (34) are exact when $f(x)$ is any linear function of x, equations (37) are exact when $f(x)$ is any third-degree polynomial in x.

We will omit listing the approximations to K_{ll} and K_{lm} which replace (35) and (36) when (37) is used.

We may note that when $m = -l/2$, which would follow from the assumption that the $(1-x^2)^{1/2}$-term in (21) is negligible, equation (22) contains the kernel $[1/2(y-\eta)] + K_{ll} - {}^1/_2K_{lm}$. As

$$K_{ll} - \frac{1}{2}K_{lm} = \frac{1}{2\pi^2}\int_{-1}^{1}\int_{-1}^{1}\sqrt{\frac{1+x}{1-x}}\sqrt{\frac{1-\xi}{1+\xi}}\,K dx d\xi \quad (37)$$

equation (22) is then reduced to a combination of equations (12) and (14) of the Weissinger procedure, which is as it should be.

It is to be expected that the solution of equations (22) and (23) is nearer to the exact solution of lifting surface theory than is the solution of the combined equations (12) and (14).

An example where the system of two simultaneous equations will give a meaningful answer whereas the one-equation procedure will not is given by a wing formed such that $w = \text{const.}\,\partial h/\partial x = C(y)(1-2x)$, that is, a wing for which according to the two-dimensional theory every section has zero lift but non-zero moment. The one-equation procedure predicts that the effect of finite span does not modify the zero lift property at every spanwise station. It says nothing about the effect of finite span on the moment distribution. The present two-equation procedure permits

determination of the spanwise variation of moment distribution and indicates that the effect of finite span modifies the zero lift properties which the two-dimensional theory predicts.

It is thought that the idea underlying the proposed two-equation procedure, which may be extended to an n-equation procedure, will also be useful in other problems of lifting-surface theory.

Summary.—The steady motion of a rectangular lifting surface in incompressible flow is considered, with the following two objectives. (1) A modified derivation is given of Weissinger's improvement of Prandtl's lifting-line theory. (2) The integral equation of lifting-line theory (and Weissinger's improvement) for the spanwise variation of lift intensity is generalized to two simultaneous integral equations for lift intensity and moment intensity. In so doing certain difficulties of lifting-line theory with reference to behavior at and near tip sections are substantially ameliorated. The two-equation procedure of this paper is shown to be applicable to a problem which is outside the scope of the lifting-line equation.

* This paper is based on an investigation which was carried out for Chance Vought Aircraft, Division of United Aircraft Corporation, under sponsorship of the Bureau of Aeronautics, U.S. Navy.

[1] Weissinger, J., "The Lift Distribution of Swept Back Wings," NACA T.M. No. 1120, March, 1947 (Translation of German ZWB F. B. 1553 (1942)).

[2] See for instance reference 1.

RESPONSES OF THE PROXIMAL RETINAL PIGMENT OF THE ISOLATED CRUSTACEAN EYESTALK TO LIGHT AND TO DARKNESS

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The possibility of an endocrine factor in regulating the photomechanical changes of the pigment cells of the crustacean retina was demonstrated by Kleinholz (1934, 1936) who showed that extracts of the eyestalks of *Palaemonetes* when injected into dark-adapted individuals brought about a movement of the retinal pigments to the position characteristic of the light-adapted retina. These hormonal effects have since been confirmed by Welsh (1939) for *Cambarus* and by Nagano (1947) for *Paratya*. Smith (1948) has recently reported similar effects on the retina of brachyuran crustaceans but has not detailed the types of pigment cells concerned since his observations were made indirectly on the basis of disappearance of "glow" after such injections. It was further shown by Welsh (1941) and by Smith (1948)

that the sinus gland within the eyestalk is the major source of the activity affecting these retinal pigments.

Some differences in the response of the retinal pigments to injected eyestalk extract have been observed in the different species of crustaceans investigated. The prawns studied by Kleinholz and by Nagano showed movement of the distal and of the reflecting pigments into the light-adapted position after injection of eye-stalk extract into dark-adapted individuals; the proximal pigment, however, remained dark adapted. In *Cambarus* Welsh (1939) found that the proximal pigment had a higher threshold than the distal pigment for the injected eye-stalk extract, because when more concentrated extracts were used, the proximal pigment also moved into the light-adapted position.

As has already been pointed out (Kleinholz, 1942, 1947) conclusive proof of endocrine regulation of the photomechanical changes of the retinal pigment cells would be demonstrated, according to conventional endocrine procedure, by experiments complementing those described above. That is, if injection of sinus gland extract causes light adaptation of a retinal pigment, then surgical removal of the sinus gland should result in the dark-adapted phase of that pigment, or should result in that pigment's being unable to move into the light-adapted position under conditions which ordinarily bring about light adaptation.

In attempting to resolve this point regarding the proximal pigment of the crayfish, experimental results were obtained that indicated the activity of this retinal pigment was more complicated than was originally suspected, and that possibly more than a single mechanism of control may be concerned in regulating its activity. The effect of surgical removal of the sinus glands from the eyestalks of the crayfishes, *Cambarus* and *Astacus*, on the ability of the proximal pigment to respond to light and to darkness has been described in preliminary reports (Kleinholz, 1947, 1948). In these animals it was demonstrated that apparently normal light and dark adaptation of the proximal retinal pigment occurred after sinus gland removal. The conclusion reached in the 1948 account was that, in addition to regulation of the proximal pigment by the sinus gland, other regulatory sources of endocrine secretion outside of the sinus gland might be present, as well as the possibility that the proximal pigment cells of the crayfish retina could respond directly to light and to darkness.

The experiments reported here were performed with four groups of the crayfish, *A. trowbridgei*. Two of these groups were initially dark adapted in a photographic dark room, while the remaining two groups were initially light adapted by keeping the animals in a brightly illuminated environment. In the first of these light-adapted groups, one eyestalk was carefully ablated from each animal in the group, placed in a moist chamber and the isolated stalks placed in darkness; the second eyestalk of each animal

in the group was then excised and fixed, to verify the degree of light adaptation of the retinal pigments at the time of ablation of the experimental eyestalk. After the isolated eyestalks had been in darkness for 3 to 5 hours they were fixed. Both the experimentally isolated eyestalks and the control eyestalks were subsequently sectioned and the position of the pigment cells examined histologically. The proximal pigment of the isolated retinas was in the position typical for dark adaptation, while that of the control eyestalks was typically light adapted.

The second group of light-adapted crayfish was used as a control for the procedures employed with the first group. In this group the isolated eyestalks were placed in moist chambers and kept on an illuminated white background; the second eyestalk of each animal was fixed as before. The ablated eyestalks were fixed after illumination for 3 to 5 hours. Examination of the sectioned retinas showed that the proximal pigments of both sets were completely light adapted.

Similar experiments were performed with the two groups of dark-adapted *Astacus*. In the first group one eyestalk from each animal was removed (in about 30 seconds) by red light from a photographic safelight, and, after being put in the moist chamber, was placed on an illuminated white background. The second eyestalk of each animal was immediately fixed as a control while the isolated stalks were fixed after 5 hours of illumination. Histological examination revealed the proximal pigment of the isolated eyestalks to be light adapted, while the control retinas were dark adapted. The second group of dark-adapted animals served as control for the methods used. In this set, both the isolated eyestalks (which had been put in moist chambers and maintained in darkness for about 5 hours) and the second eyestalk of each animal showed the proximal pigment in the position characteristic for the dark-adapted retina.

The evidence of the above experiments indicates that proximal retinal pigment cells can respond directly to light and to darkness while isolated from the central nervous system (except for the optic ganglia) and from the circulatory system. A more detailed account of these and of other experiments indicating the rôle of the nervous system in retinal pigment migration will be reported later.

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THE SURFACE GEL LAYER OF FUNDULUS EGGS IN RELATION TO EPIBOLY

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There have been several studies relating the properties of the surface gel layers of Amphibian[1] and Teleost[2] eggs to the movements of gastrulation. Teleost eggs, with their remarkable epiboly, offer excellent material for a further analysis of this question. The present report outlines certain preliminary studies on the properties of the surface gel layer of the eggs of *Fundulus heteroclitus*, with special reference to its possible rôle in epiboly.

All studies were made on eggs from which the chorion (or "shell") had been removed. Unless otherwise stated, unbuffered double-strength Holtfreter's solution was the medium employed. It was possible to demonstrate at the outset that Fundulus eggs possess a surface gel layer, by microdissection with glass needles. This gel layer constitutes the outer coat in oviducal eggs, unfertilized eggs and in eggs activated by sea water, as well as in developing eggs at all stages as far as closure of the blastopore. It is continuous, non-pigmented and constitutes the outer layer of both the unsegmented yolk sphere and the blastodisc. The outer surface of this gel layer is non-adhesive. If a blunt instrument is pressed against the egg, the gel layer shows resistance and is thrown into radiating folds. Microdissection of all stages through blastopore closure (Oppenheimer, stage-15)[3] indicates that the surface gel layer is the outer layer of the epiblast to which all epiblast cells are attached. Thus the epiblast tends to come off in sheets when removed. Furthermore, the gel layer of the epiblast is continuous with that of the yolk.

Properties of the Surface Gel Layer.—When a wound is made in the gel layer of the yolk sphere, the aperture first becomes round and expands in size as the gel layer retracts, presumably because of the tension resulting

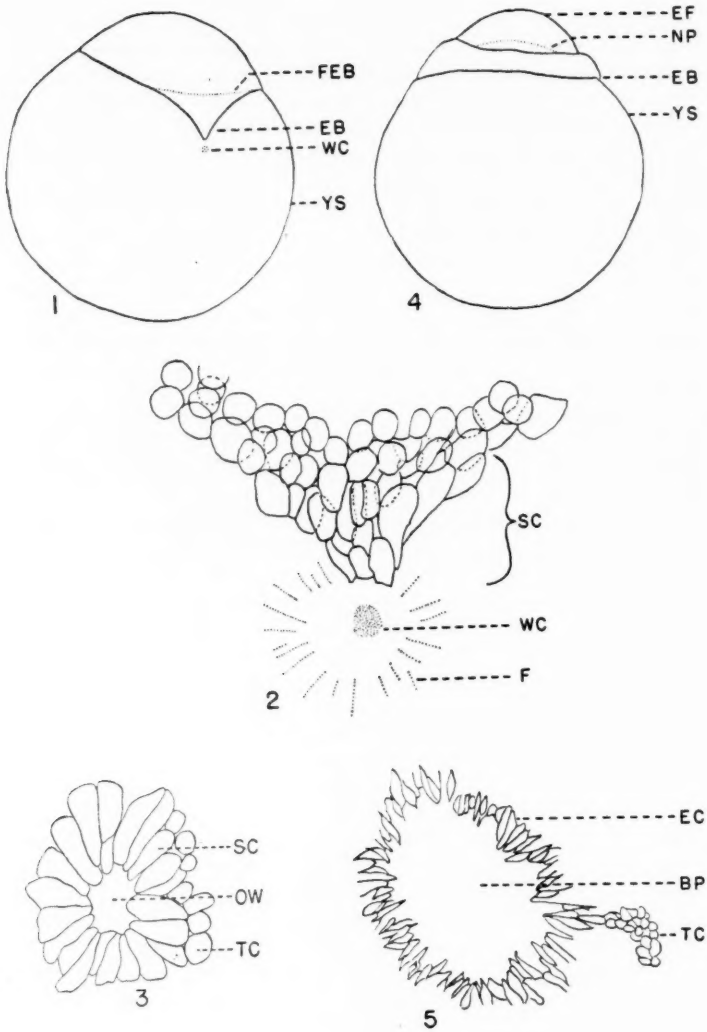
from the turgor of the yolk. This rounding up and expansion of the wound also indicates that the gel layer possesses elastic properties. After this initial increase in size the aperture is closed rapidly, while the relatively fluid yolk continues to ooze during all but perhaps the final stages of the process. This whole process takes from 2–5 minutes depending upon the size of the wound. As the wound closes, folds or stress lines appear in the gel layer, radiating from the wound. The gel layers of oviducal eggs and unfertilized eggs activated by sea water have the same ability to close wounds rapidly. This process takes place in typical fashion when the eggs are in single, double or quadruple-strength Holtfreter's solution, in sea water and in distilled water.

In order to ascertain whether wound closure is actually due to contraction of the surface gel layer, carbon marks[4] were placed on opposite edges of an open wound (prior to the onset of wound closure). As the wound closed, the marks were drawn toward each other until they almost met at the point of wound closure. Such an experiment indicates that wound closure is chiefly the result of the contraction of the edges of an already existing surface layer, rather than the formation of a new membrane from the yolk cytoplasm. The very last stage appears to be a process of protoplasmic "clotting," wherein a small amount of yolk cytoplasm coagulates and forms a plug.

If carbon marks are made at various points on the surface of one hemisphere of the egg and a wound then made in the midst of these marks those marks nearest the closing wound move toward each other but the distance between these marks and marks further away increases. Thus the surface layer stretches or expands as the process of wound closure proceeds. It seems also from this experiment that a large part, if not the total, surface layer of the egg is involved. The integrity of the entire surface layer is not necessary for wound closure, however, inasmuch as two or more wounds in the surface layer will close at the same time, in typical fashion, even though one of the wounds is quite large, e.g., 1 mm. in diameter, which is one-half the diameter of the entire egg.

Wound closure in the surface gel layer of the yolk sphere near the edge of the blastoderm, at all stages from the one-celled blastodisc to the closure of the blastopore, results in a stretching of the blastoderm in the direction of the closing wound, and also in a stretching of individual adjacent cells and periblast nuclei as well. This phenomenon (see Figs. 1 and 2), takes place in a few minutes' time. It demonstrates that the cells at the edge of the blastoderm (and nuclei of the marginal periblast) are attached to the surface gel layer of the yolk sphere in such a manner that tension in this layer exerts a corresponding tension on them, resulting in an appropriate distortion of cell shape.

Experimental demonstration that the surface gel layer is continuous


FEB
EB
WC
YS
1
EF
NP
EB
YS
4
SC
WC
F
2
SC
OW
TC
3
EC
BP
TC
5

over the entire surface of the blastoderm is provided by further wound healing experiments. If a group of cells is removed from the epiblast layer in a blastula or gastrula stage, a process of wound closure is initiated, which appears similar to that in the yolk surface layer, although it is slower, taking 7 to 12 minutes or longer. It results in a radial stretching of the cells adjacent to the wound. This stretching becomes apparent when the wound is approximately half closed, and is continually augmented until the completion of the process (see Fig. 3). Such a radial stretching effect is clear evidence that a supra-cellular force, presumably the surface gel layer, is concerned in wound closure in the epiblast cell layer.

A wound in the surface of one of the blastomeres in an early cleavage stage is rapidly closed in a similar manner, with resulting radial folds or lines of stress in the blastomere surface. Apropos of wound healing in early blastomeres, it might be mentioned that it is relatively difficult to wound the surface of a blastomere of the 1- or 2-cell stage. The surface seems tougher than that of the yolk. Furthermore, since little or no cytoplasm pours out of the wound, the cytoplasm of these cells must have a higher viscosity than the yolk.

As a result of these various wound healing experiments it is evident that the outer surface layer of Fundulus eggs is a gel layer possessed of marked elastic properties. In this respect the surface gel layer of Fundulus eggs is like that of Amphibian and trout eggs (cf. Holtfreter[1] and Devillers[2]). However, if rapidity of wound closure be an adequate criterion,

EXPLANATION OF PLATE

(Drawings based on camera lucida tracings)

FIGURE 1

Stretched edge of blastoderm resulting from wound closure in surface gel layer of yolk sphere in Oppenheimer stage 10. × 20.

FIGURE 2

Stretched cells at the edge of the blastoderm resulting from wound closure in the surface gel layer of the yolk sphere. Stage 8. × 100.

FIGURE 3

Stretched cells of the epiblast after partial wound closure. Stage 11. × 220.

FIGURE 4

Protrusion of exposed floor of blastocoel, after removal of roof of blastocoel. Stage 10+. × 20.

FIGURE 5

Elongate cells at lips of blastopore in normal embryo. Stage 15. × 100.

BP, blastopore; *EB*, edge of blastoderm; *EC*, elongate cells at lips of blastopore; *EF*, exposed floor of blastocoel; *F*, radiating folds in surface gel layer; *FEB*, former edge of blastoderm; *NP*, normal position of floor of blastocoel; *OW*, open wound; *SC*, stretched cell; *TC*, typical epiblast cell; *WC*, point of wound closure; *YS*, yolk sphere.

the elasticity of the Fundulus egg gel layer is considerably greater than that of Amphibian and trout eggs.

The floor of the segmentation cavity or blastocoel was studied in late cleavage and blastula stages to see if it likewise possesses such an elastic surface layer. When a large section of the roof of the blastocoel is removed, the floor of the blastocoel is exposed, consisting at this stage solely of the surface of the yolk sphere. It is not yet covered by periblast. Within a few minutes after such an operation, the exposed surface bulges out as shown by figure 4. This demonstrates both the turgor of the yolk, and the lack of elastic strength in the surface of the yolk at the floor of the segmentation cavity. When a wound is made in the yolk surface at the floor of the segmentation cavity it gapes and remains open. The relative ease with which such a wound is made itself indicates the weakness of this layer. It is thus apparent that the yolk surface at the floor of the segmentation cavity in early stages lacks a surface layer with the same elastic, contractile properties as are possessed by the peripheral surface of the egg. Similar wounding experiments on the floor of the blastocoel in late blastulae and all stages of gastrulae, when the floor is covered with periblast, suggests that the surface at these stages possesses an elastic gel layer, inasmuch as wound closure seems more typical, although often slower (ca. 10 min.).

If an early blastula with an open wound in the floor of the blastocoel remains in the bottom of a dish for an hour or so, all of the yolk oozes out, and all that remains is the surface gel layer lying in the bottom of the dish with marginal blastoderm cells clinging to the edges of the aperture. It has the appearance of an empty cellophane bag. This forms superb material for physicochemical studies. A few preliminary experiments have been done along these lines.

Mechanical and Chemical Studies.—The gel layer, in this isolated condition, will remain intact and apparently in a normal state for at least 12 hours in double-strength Holtfreter's solution. It is non-adhesive on its outer surface (as in the intact egg) and sticky and viscous on its inner surface. If the inner surfaces of 2 pieces are pressed together they will adhere quite firmly. The elastic properties of the material can be demonstrated by stretching pieces between two glass needles. In this manner a piece may be stretched to half again its length, returning to its original length upon release. Small pieces of the gel layer, with cells clinging to them, tend to round up into spherical globules. When the gel layer is emptied of yolk and, therefore, in a slack condition, a wound in it no longer expands into a round opening in the usual manner. Apparently, the normal process of wound closure in the surface gel layer is dependent upon a state of tension in the gel layer, resulting from the normal turgidity of the egg. After a certain small amount of mechanical manipulation, the isolated gel layer completely solates. This is a property of thixotropic

gels. It does not return again to the gel state, however, upon standing, as is typical of these gels.

In Ca^{++}-free sea water the surface gel layer of the intact egg dissolves, or better—solates, after the egg has been in the medium for 1–2 hours. In some cases the egg first flattens out on the bottom of the dish like a thick pancake, suggesting a general thinning and weakening of the gel layer, prior to solation. In other cases, the egg retains its spherical shape and its normal wound healing ability for approximately $1^1/_2$ hours. At about this time, however, a wound forms readily without extreme intervention and remains open. All of the surface gel layer then solates in the course of a few minutes. At no time in any of these cases was the outer surface observed to become sticky, as is normal for the inner surface. Both development and wound closure of Fundulus eggs take place normally in distilled water. The effect of Ca^{++}-free sea water is, therefore, not due simply to a lack of Ca^{++}-ions. The positive action of the monovalent ions in the ionically unbalanced solution must also be considered.

The behavior of the surface gel layer of the Fundulus egg in Ca^{++}-free sea water is characteristic of the behavior of the surface gel layers of other eggs in Ca^{++}-free media, e.g., Amphibian. The hyaline plasma layer of Echinoderm eggs also solates in Ca^{++}-free sea water. This latter fact suggests that the similarities of the surface gel layer and the hyaline plasma layer may be more than superficial, a subject which invites further investigation.

Possible Role of Surface Gel Layer in Epiboly.—The properties of the surface gel layer, as outlined in the previous pages, suggest that it may be of some importance in relation to the movements of gastrulation. It certainly seems probable that the surface gel layer, being the outer layer of the epiblast to which all epiblast cells are attached, acts as a coördinating and unifying force in the highly integrated cell movements characteristic of the epiblast in Teleost gastrulation. Furthermore, the stretching effect of the gel layer upon the edge of the blastoderm in wound healing is especially suggestive of a possible causal rôle of the gel layer in epiboly. To test this idea, detailed observations were made of cell shape at the edge of the blastoderm in all stages of epiboly (in *normal* eggs). At the stage when epiboly *seems* to proceed at the greatest rate, namely the final closure of the blastopore (stage-14+ to stage-15), there is a radial elongation of the cells at the lips of the blastopore (see Fig. 5). These elongate cells appear to be stretched toward the point of blastopore closure. This suggests that the blastoderm is being pulled over the surface of the yolk by the contractile tension of the surface gel layer of the yolk sphere. If this be the case, one should be able to cause a uniform stretching of cells at the lips of the blastopore in an earlier stage, e.g., stage-13+ or stage-14, by making a wound in the surface gel layer at the point of future blastopore

closure. When this was done, cells at the edges of the blastoderm became stretched in the direction of the point of closure of the blastopore. Furthermore, it was possible by such an experiment to cause precocious closure of the blastopore with the embryo only in an embryonic shield stage (stage-13+).

One further case supports this possible rôle of the surface gel layer in epiboly. In the series of experiments in which the roof of the blastocoel was removed during a late blastula stage (stage-11) one egg remained intact over an 18-hour period. In spite of the fact that the large wound in the roof of the blastocoel remained open, epiboly took place completely with resulting closure of the blastopore. Such a result constitutes supporting evidence for the thesis that epiboly is not an overgrowth, as has often been thought, but is rather the result of the blastoderm being pulled down over the yolk.

If the hypothesis is true that epiboly is due to the contractile tension of the surface gel layer of the yolk sphere on the cells of the blastoderm, it is likely that in the course of epiboly there would be a greater stretching of the blastoderm nearest its edge, with gradually decreasing amount of stretching as one proceeds toward the animal pole, assuming that the surface gel layer of the blastoderm has uniform elasticity. It is certainly true, for example, that, after the closing of a wound in the surface gel layer of the yolk sphere, only the marginal blastoderm cells, nearest the closing wound, show marked stretching.

To check this point, the relative degree of stretching of different regions of the blastoderm was studied by the method of carbon marking. Marks were placed at various points on the surface of the epiblast in an early gastrula stage. Camera lucida records of the positions of these marks relative to one another were made every few hours during the course of epiboly. The distance between carbon marks over the entire blastoderm increased uniformly, as epiboly proceeded. This sort of result suggests that there is a uniform expansion, or spreading, of the epiblast, and, therefore, also of the surface gel layer of the blastoderm, in epiboly. Such a phenomenon indicates that epiboly may not be simply the result of tensions exerted on the edge of the blastoderm by the surface gel layer of the yolk sphere. The situation is more complex, it would seem, than some previous workers have been willing to admit (e.g., Lewis, who, however, quotes no experimental evidence). It is, of course, possible that any tension exerted on the edge of the blastoderm during epiboly is too small to be detected by the carbon marking technique. Nevertheless, these data indicate that the expansion of the surface gel layer of the epiblast must also be considered as a possible causal factor in epiboly.

In spite of these suggestive observations and experimental results, it is still an open question as to whether the surface gel layer actually plays

a determining rôle in epiboly in the Fundulus egg. If it is causally important in epiboly, the precise rôle it plays has not yet been determined. Further investigation of the relation of the surface gel layer to the cell movements of early morphogenesis is necessary, for it may possibly shed some light on one of the more obscure aspects of causal embryology, the mechanics of gastrulation.

It should be emphasized that this report has been concerned exclusively with studies on eggs of *F. heteroclitus*. The results are not necessarily representative of Teleost eggs in general. In fact, the surface layers of the eggs of some forms, e.g., *Platypoecilus maculatus* and *Lebistes reticulatus*, seem to have a much lower degree of elastic strength than that of Fundulus eggs. The surface layer of Toadfish eggs (*Opsanus tau*) and Medaka eggs (*Oryzias latipes*), however, appear to be more like that of the Fundulus egg. The properties of the surface gel layer suggest that one of its functions may be to protect the egg from the effects of osmotic changes (as pointed out by Devillers) and mechanical buffeting. The weakness of the gel layers of Lebistes and Platypoecilus eggs thus may be related to the viviparity of these forms, and, conversely, the strength of the gel layers of Fundulus, Opsanus and Oryzias eggs to oviparity.

Summary.—Fundulus eggs possess a surface gel layer, which constitutes the continuous, outer layer of both the unsegmented yolk sphere and the blastoderm. It has striking elastic properties and plays a decisive rôle in wound healing. The contraction of the surface gel layer in wound closure stretches adjacent cells and pulls a large sector of the blastoderm toward the wounded area. At closure of the blastopore in normal embryos, the cells at the blastopore lips elongate. At an earlier stage, this phenomenon is simulated and blastopore closure hastened if the surface gel layer is wounded at the point of future closure of the blastopore, suggesting that epiboly may be the result of contractile tension exerted by the surface gel layer of the yolk sphere. The behavior of the blastoderm, as observed in marking experiments, indicates that the spreading of the surface gel layer of the blastoderm must also be considered as a possible factor in causing epiboly.

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